ORIGINAL ARTICLE

Physico-Chemical Characterization and Acute Toxicity Study Ethanolic Extract of *Parthenium Hysterophorus*

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Abstract

Objectives: *Parthenium hysterophorus* is an invasive herbaceous plant and considered as noxious weed. Sesqueterpines lactones which are responsible for allergenic nature cause various serious illness ranges from allergy rhinitis to asthma. Physico-chemical Characterization and acute toxicity study of plant data support to find suitable solution to overcome the exposures and draw medicinal value of uncontrollable spreading weed. **Materials and Methods:** Ethanolic extractof *P. hysterophorus* purchased from AMSAR Pvt. Ltd., Indore, Madhya Pradesh, and perform organoleptic characterization, solution stability, powder bulk characterization, extract microscopy (Labomed Lx 400) at ×40. Phytochemical screening and Infra-red spectrum (Fourier transform infrared spectroscopy, Shimadzu) determine also perform protein estimation using folin-lowry method and acute toxicity study on 06 albino rats on three different reported concentrations. **Results:** The powdered extract bulk density 0.6 g/cm^3, Tapped density 0.86 g/cm^3 and angle of repose is recorded 38° which reflects the powdered extract flow falls in fair to passable range. In the screening the main phyto-constituents observed presence of sesqueterpines lactones, alkaloids carbohydrate, flavnoidsand saponins, in the microscopy study presence of pollens, pollen sacs, corolla and trichomes. Acute toxicity study of ethanolic extract of *P. hysterophorus* showcase toxic symptoms includes Food avoidance, Sedation and minimum movement, Irritations and restlessness observed respective to dose found lethal dose (LD_{50}) 600 mg/kg in albino rats. Protein estimation observed 90 µg/mL in 10% sample solution. **Conclusion:** Characterization of ethanolic extract of *P. hysterophorus* provide insight related to presence of allergen protein Par-H-1 and LD_{50} in albino rats, these data can be use to troubleshoot the management of disease related to weeds such as seasonal pollen allergies. More detail chemical analysis also requires enhancing understanding the allergen nature of the weed.

Key words: Acute toxicity study, ethanolic extract, lethal dose$_{50}$, sesqueterpines lactones

INTRODUCTION

There are some invasive species of weeds which are un-controllably spreading in India also in the world. Management of these weeds is a biggest question for the society because these are not only dangerous for agriculture but also are harmful to human as well as animals. *Parthenium hysterophorus* is one of that species belongs to *Asteraceae* family and commonly found in Asia, Africa and Europe. Some literature suggested the invasion of this weed in India found to be late 80's and till date government not have a full proof weed management procedure for this plant. *P. hysterophorus* also known as congress grass, carrot grass, santa maria feverfew, famine weed and whitetopweed found difficult to manage by pesticides that's why government of India,department of agriculture and farmers welfare starts a campaign "uproot and burn the weed" especially to control the over spreading the pollens of Parthenium plant.[1,2]

Dermatitis, pollen allergies, bronchitis, asthma and various chronic and severe diseases results of air borne pollen of *Asteraceae* family plants. A single plant can produce approximately 20,000 pollen and also able to re-grow after completion of life span which is 8–10 years. Sesqueterpines lactones mainly Parthenin is mainly responsible of allergies.[3,4]

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Acute toxicity as lethal dose (LD_{50}) is an important parameter as plant extract characterization supports various researches and suggest the better management approaches, research outcomes and awareness proofs. *P. hysterophorus* ethanolic extract by oral route calculated using up and down method and found 676.65 mg/kg on albino rat and symptoms observed like labored breathing, tremor, abnormal gait, food avoidance, and over urination.[5]

MATERIALS AND METHODS

Freeze dried ethanolic extract of *P. hysterophorus* purchased from AMSAR Pvt. Ltd. Indore and physico-chemical characterization performed to observe the bulk characterization, phytochemical identifications, infra-red (IR) spectroscopic analysis, protein estimation and acute toxicity on albino rats.

Organoleptic characterization

Sensory characterization such as physical appearance, color, odor, and taste performed to know impact of material physical appearance on finished formulation.

Solution stability (open exposure)

1 g powdered extract suspended in 100 mL purified water poured in petri dish as open exposure at room temperature for 15 days.

Bulk characterization

To understand the physical characteristics of freeze dried powdered extract, powder bulk characterization such as densities, carr's index, hausner's ratio, angle of repose, and particle size analysis performed using microscopy method.[6]

Powder microscopy

Freeze dried ethanolic powdered extract passed through 60# sieve and performed microscopy to understand the structural and functional properties using Labomed LX400 microscope on ×40.[7]

Phyto-constituents analysis

2 g sample in 200 mL purified water to prepare test solution and phytochemical analysis performed by following test.[8,9]

IR analysis for identification

Functional group determination data support the identification and authentication of plant extract, Infra red analysis performed using shimadzu Fourier transform infrared spectroscopy (FTIR) at 4 (1/cm) and compared the graph against Parthenin standard structure.[10]

Table 1: Procedure phytochemical analysis

S. No.	Test	Procedure
1	Wagner reagent (for Alkaloids)	Alcoholic solution of iodine in potassium iodide
2	Hager reagent (for Alkaloids)	Glucose+Picric acid→Gluconic acid+Picramic acid
3	Molish test (for carbohydrates)	Naphthol+sulfuric acid
4	Baljet test (for sesquiterpenes lactones)	Picric acid+sodium hydroxide
5	Sulfuric acid test (for Flavnoids)	Concentrated Sulfuric acid was added in sample appearance of yellow–orange colors
7	Foam test (for Saponins)	2 mg of sample in 1 mL of purified water S mixture was shaken vigorously for 1 min.
8	Wagners test (for Alkaloids)	Alcoholic solution of iodine in potassium iodide

Table 2: Solution preparation for protein estimation by Lowry's method

Solution	Procedure
A	2% (w/v) sodium carbonate in 1 N sodium hydroxide
B	1% (w/v) copper sulphate
C	2% (w/v) sodium potassium tartrate
D	Copper reagent- Mix 0.5 volume of solution B, 0.5 volume of solution C and 50 volumes of solution A
E	Standard solution of BSA 30 mg % (30 mg in 100 mL)
F	Folin–Ciocalteau reagent

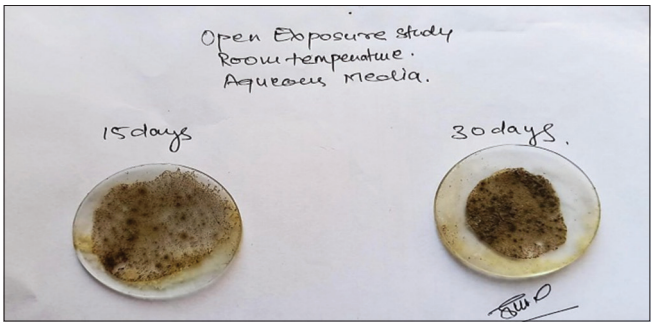

Figure 1: Solution stability in open exposure

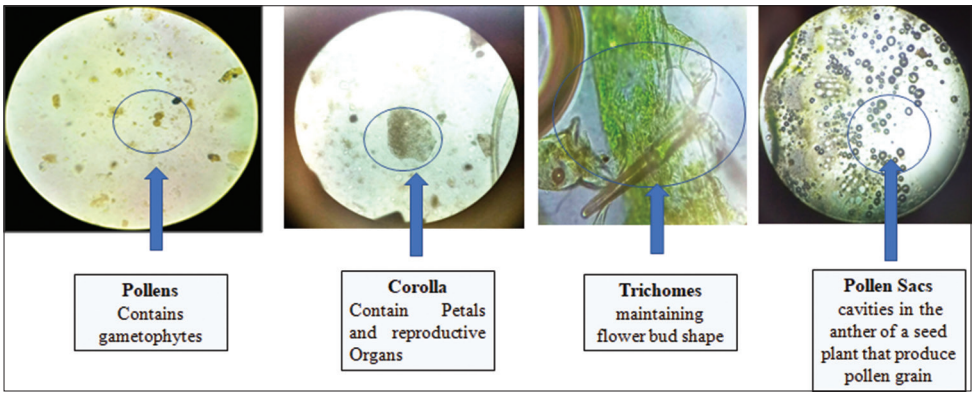

Figure 2: Powder microscopy of *Parthenium hysterophorus* ethanolic extract

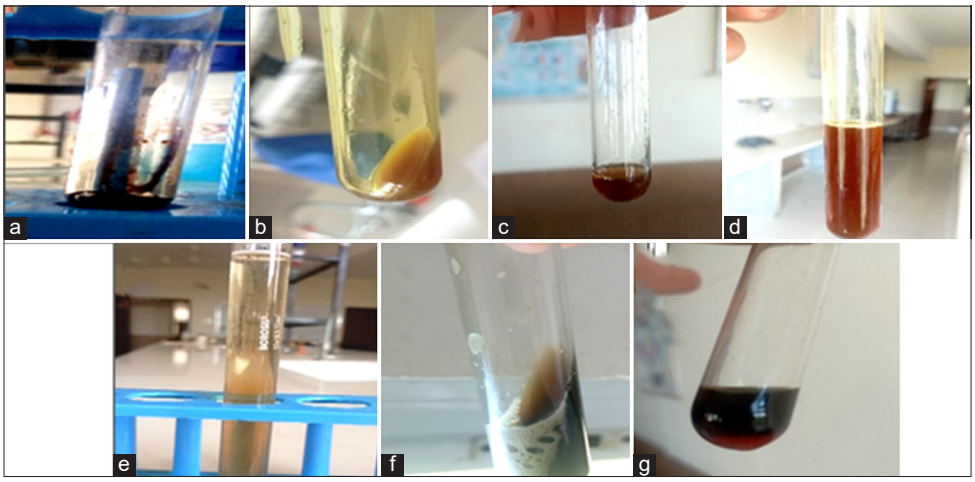

Figure 3: (a-g) Images of Phyto-constituents observations

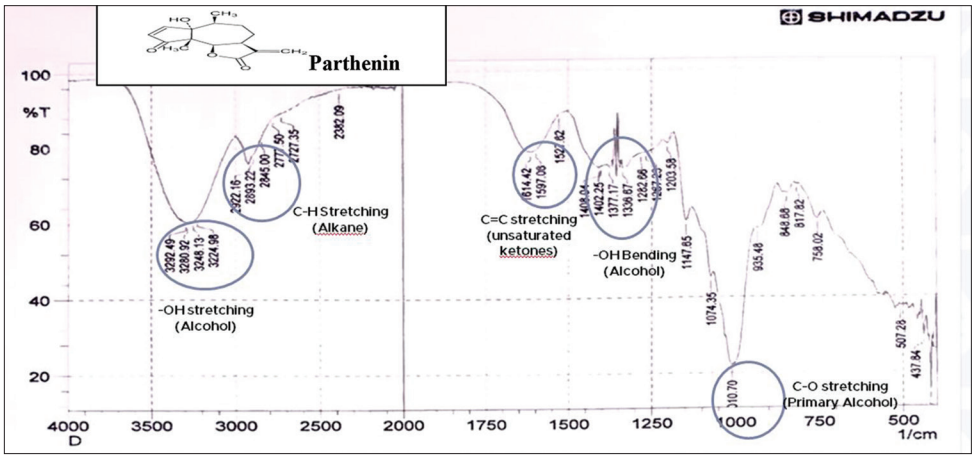

Figure 4: *Parthenium hysterophorus* extract infra-red spectroscopy

Protein estimation using Folin-Lowry method

Solution preparation

After sample preparation dilutions of 60, 90, 120, 150, and 180 µg/mL, analyze the dilutions in colorimeter at 680 nm.[11]

Acute toxicity study on albino rat

Healthy adult male or female albino rat weigh and assign a number to each and keep in separate cage at room temperature. Keep animal on fasting (without food and water) for aprox 14–16 h before dosing. Prepare sample solution of ethanolic extract of *P. hysterophorus* according

Table 3: Dose calculation for acute toxicity study as per body weight

Group code	G1	G2	G3	Identification
Dose to each group	**400 mg/kg**	**600 mg/kg**	**800 mg/kg**	
Weight of Animal in each group (in g)	88	125	130	With mark on tail
	109	107	122	Without mark on tail
Dose to be administer orally (in mg)	17.6	50	104	With mark on tail
	21.8	42.8	97.6	Without mark on tail
Stock to be administer orally (in mL)	0.6	1.7	3.4	With mark on tail
	0.7	1.4	3.2	Without mark on tail

Table 4: Organoleptic characterization

S. No.	Test	Observation
1	Description	Dark green-colored fine powder
2	Odor	Musky and earthy
3	Taste	Very bitter in taste
4	Nature	Hygroscopic
5	Texture	Fine powder

Table 5: Bulk characterization

Powder characteristics	Observation	Flow property
Bulk density	0.62 g/cm^3	-
Tapped density	0.86 g/cm^3	-
Carr's index	27%	Fair to passable
Hausner's ratio	1.33	Passable
Angle of repose	38°	Passable

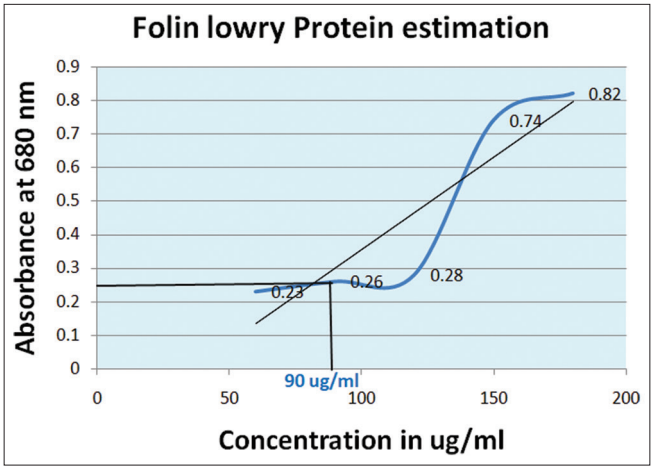

Figure 5: Protein estimation graph

to 200 mg/kg, 400 mg/kg and 600 mg/kg body weight in distilled water. Observe each group in interval of 1, 3, 7, and 14 days for any toxic symptoms or lethality.[12]

Toxic symptoms includes:

- Food avoidance
- Irritations and restlessness
- Sedation and minimum movement
- Diarrhea
- Abnormal gait or any other.[13]

Dose calculation

Stock preparation: 300 mg extract in 20 mL purified water
Strength: 30 mg/mL or In 1 mL = 1/30 = 0.3333 mg
Multiplying factor: 0.033 mL/mg
Animal species: Albino rat
Total number: 06 (3 groups/2 in each group).

RESULTS

Organoleptic characterization

The Organoleptic characterization of powdered extract performed and observation mentioned in Table 4 (Organoleptic characterization).

Solution stability (open exposure)

Fungus observed in extract suspension in 15 days and completely dried out in 30 days as open exposure at room temperature.

Bulk characterization

Bulk characterization is an important data in case of powdered drug, characterization performed and data mentioned in Table 5 (Bulk characterization).

Powder microscopy

Freeze dried powdered extract passed through 60# sieve to perform microscopy.

Phyto-constituents analysis

The qualitative estimation of phyto-constituents provides and presence and absence of phytochemicals and powdered extract, test perofmed and observations mentioned in Table 6 (Phyto-constituent observations).

Table 6: Phyto-constituent observations

S. No.	Phyto-constituent	Test	Observations	Inference	Image code
1	Alkaloids	Wagner's test	Insoluble purple color precipitate	Present	a
2	Alkaloids	Hager's test	Yellow precipitate	Present	b
3	Carbohydrate	Molish test	Purple color ring	Present	c
4	Sesqueterpines lactones	Baljet test	Orange or red colors	Present	d
5	Tannins	Ferric chloride test	Blue color	Present	e
6	Flavnoids	Sulphuric acid test	Yellow-orange colors	Present	f
7	Saponins	Foam test	Stable foam formed	Present	g

Table 7: Extract infra-red spectroscopy peak comparison

Characteristic peak	Class	Peak detail	Parthenin standard peaks (cm^{-1})	Observed extract peaks (cm^{-1})
O-H stretching	Alcohol	Strong, broad	3200–3550	3280
C-H stretching	Alkane	Medium	2840–3000	2893
C=C stretching	α,β-unsaturated ketone	Strong	1610–1620	1614
O-H bending	Alcohol	Medium	1330–1420	1402
C-O stretching	primary alcohol	strong, sharp	1000–1085	1010

Table 8: Protein estimation observation by Folin Lowry method

S. No.	Volume of standard	Volume of distill water	Concentration of standard soln in Ug/mL	Folin I	Lowry II	Absorbance at 680 (red filter)
1	0.2	0.8	60	5 mL	0.5 mL	0.23
2	0.3	0.7	90	5 mL	0.5 mL	0.26
3	0.4	0.6	120	5 mL	0.5 mL	0.28
4	0.5	0.5	150	5 mL	0.5 mL	0.74
5	0.6	0.4	180	5 mL	0.5 mL	0.82
6	Unknown	-	-	5 mL	0.5 mL	0.26

IR analysis for identification

P. hysterophorus pure extract spectra has peaks at 3,299 cm^{-1}, 1,632 cm^{-1}, 1,362 cm^{-1}, and 1,222 cm^{-1}. These peaks are attributable to broad O-H, C=O, and C-N stretching bands characterized by phenols, flavnoids, and amine metabolites in the plant extract.

Protein estimation

Par-H1 protein estimation by Folin Lowry's method observation.

Acute toxicity study

Weighing and marking of animals

A Statistical tool Probit Analysis used to determine acute toxicity data mentioned in Table 10 (Probit analysis table for LD50 calculation).

Acute toxicity observations

Correction factor (already mentioned below table) For 0% dead=100 (0.25)/n, = 100 (0.25)/2=12.5. *For 100% dead=100 (n 0.25/n) = 100 (2 0.25/2) = 87.5.

Probit analysis for LD_{50} calculation

Albino rat divided into groups based on weight and dose calculated accordingly observation mentioned in Table 3 (Dose calculation for acute toxicity study as per body weight).

Correction factor for 0 % and 100 % dead

After dose rats observed for up to 14 days and observation mentioned in Table 9 (Acute toxicity observations up to 14 days of dosing).

Table 9: Acute toxicity observations up to 14 days of dosing

Observation period	G1 (400 mg/kg)		G2 (600 mg/kg)		G3 (800 mg/kg)	
	Mark	Without mark	Mark	Without mark	Mark	Without mark
Immediate after 30 min of dosing	Excessive thirst (Polydipsia), irritation and redness on nose, paw, genitals, and head also restlessness observed in all animals of all groups					
After 1 h of dosing	Less irritation and restlessness	High irritation on face, ear and paw	Less irritation and restlessness	Less irritation and settled	Mortality	Very less Movement and shivering
After 3 h of dosing	Less irritation and restlessness	High irritation on face, ear and paw	Mortality	Less irritation and settled	High irritation on face, ear and paw	Mortality
After 12 h of dosing	Calm and no observation			Food avoidance	Excess urination	
After 3 day of dosing	Calm and no observation			Calm and no observation	Less movement and settled	
After 7 day of dosing	Calm and no observation			Calm and no observation	Calm and no observation	
After 14 day of dosing	Calm and no observation			Calm and no observation	Calm and no observation	

Table 10: Probit analysis table for LD_{50} calculation

Group	Dose	log dose	Percentage dead	Percentage corrected	Probit
I	400	2.6	0	12.5*	0
II	600	2.77	50	50	5
III	800	2.9	100	87.5*	6.15

*For 0% dead=100 (0.25)/n, = 100 (0.25)/2=12.5. *For 100% dead=100 (n-0.25/n) = 100 (2-0.25/2) = 87.5

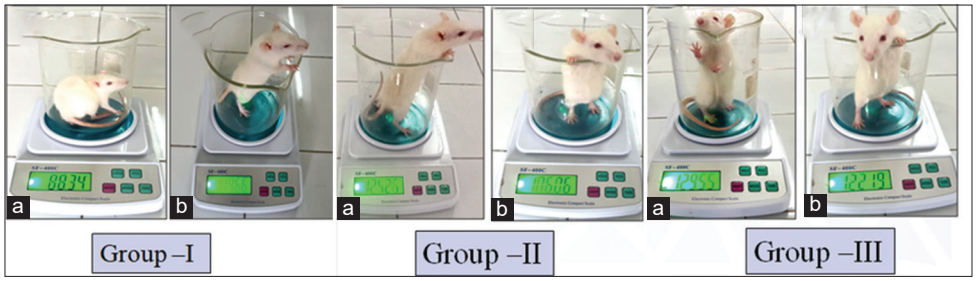

Figure 6: Albino rat weighing and marking, in (a) with mark and (b) without mark in all three Group

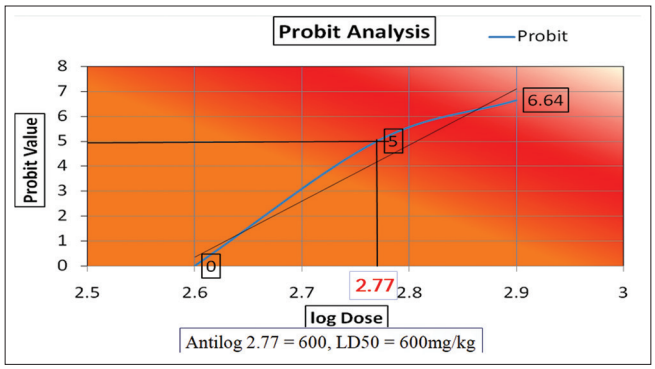

Figure 7: Lethal $dose_{50}$ calculation using probit chart method

DISCUSSION

The ethanolic extract of *P. hysterophorus* presented as a dark green fine powder with a musky, earthy odor and intensely bitter taste, reflecting its hygroscopic nature. In solution stability tests, an open aqueous suspension developed fungal growth after 15 days and fully desiccated by day 30, indicating the importance of proper storage to avoid degradation during handling or formulation. Powder flow properties—bulk density of 0.62 g/cm^3, tapped density of 0.86 g/cm^3, Carr's index of 27%, Hausner's ratio of 1.33, and angle of repose of 38°classified as fair to passable, amenable to pharmaceutical

processing with flow enhancers. Microscopy identified pollen grains, sacs, corolla fragments, and trichomes, preserving the plant's allergenic structures. Phytochemical tests verified alkaloids, carbohydrates, sesquiterpene lactones, tannins, flavonoids, and saponins, which underlie the weed's role in dermatitis, rhinitis, and asthma from pollen exposure.

FTIR spectra showed peaks at 3,299 cm^{-1} (O-H stretch), 1,632 cm^{-1} (C=O stretch), 2,893 cm^{-1} (C-H stretch), and comparable bands to parthenin standards, confirming phenols, flavonoids, and metabolites. Folin-Lowry protein assay measured about 90 µg/ml, linked to allergens like Par-h-1.[14] Acute toxicity in albino rats escalated with dose: Polydipsia and irritation at 400 mg/kg, food avoidance, sedation, and deaths at 600 mg/kg, and full lethality at 800 mg/kg. Probit analysis determined an LD_{50} near 600 mg/kg, consistent with observed symptoms such as tremors and gait abnormalities.[15]

This profile highlights the extract's challenges and opportunities, guiding strategies for allergy prevention and weed control while stressing exposure risks.

CONCLUSION

The characterization of *P. hysterophorus* ethanolic extract demonstrates a hygroscopic powder with moderate flow, abundant sesquiterpene lactones and Par-h-1 protein (90 µg/mL), and an LD_{50} of approximately 600 mg/kg in rats. The results provide actionable insights for managing the weed's public health burdens, such as pollen-induced allergies, and underscore the need for advanced studies on chronic effects and allergen mechanisms to optimize interventions.

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