e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

PHYTOCHEMICAL ANALYSIS AND HEPATOPROTECTIVE ACTIVITY OF PETROLEUM ETHER EXTRACT OF *AGERATUM CONYZOIDES*

Kiran Kushwaha*1, Varun Jain*2

*1,2SAM Global University Raisen, India.

ABSTRACT

Liver diseases represent a significant global health burden with limited effective treatment options. *Ageratum conyzoides* L. (Asteraceae), commonly known as goat weed, is a widely distributed plant traditionally used in various folk medicines for its anti-inflammatory, analgesic, and wound-healing properties. This study investigated the hepatoprotective activity and phytochemical composition of the petroleum ether extract of *Ageratum conyzoides*. Hepatotoxicity was induced in Wistar rats using carbon tetrachloride (CCl4). Liver function was evaluated by measuring serum levels of liver enzymes (AST, ALT, ALP, and GGT), bilirubin, and total protein. Histopathological examination of liver tissues was performed to assess the extent of liver damage. The phytochemical analysis was conducted using standard methods. The results demonstrated that PEEAC significantly attenuated the elevated levels of liver enzymes and bilirubin induced by CCl4, while restoring total protein levels. Histopathological findings further confirmed the hepatoprotective effect, showing reduced liver damage in PEEAC-treated groups. Phytochemical analysis revealed the presence of various bioactive compounds including terpenoids, flavonoids, steroids, and alkaloids in the petroleum ether extract. These findings suggest that PEEAC possesses significant hepatoprotective activity, possibly mediated by its antioxidant and anti-inflammatory properties due to the presence of various phytoconstituents.

Keywords: *Ageratum Conyzoides*, Petroleum Ether Extract, Hepatoprotective Activity, Carbon Tetrachloride, Liver Enzymes, Phytochemical Analysis.

I. INTRODUCTION

The liver, a vital organ, plays a crucial role in detoxification, metabolism, and storage of essential nutrients. However, it is susceptible to various injuries caused by toxins, infections, drugs, and alcohol, leading to liver diseases such as hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. Currently, conventional therapies for liver diseases are often associated with limitations and adverse effects, prompting the search for alternative therapeutic agents from natural sources.

Medicinal plants have been used for centuries in traditional medicine systems for the treatment of various ailments, including liver disorders. *Ageratum conyzoides* L. (Asteraceae), commonly known as goat weed or billygoat weed, is an annual herbaceous plant widely distributed in tropical and subtropical regions [2]. Traditionally, *A. conyzoides* has been used for its anti-inflammatory, analgesic, antimicrobial, wound-healing, and insecticidal properties [3]. Previous studies have reported the presence of various bioactive compounds in *A. conyzoides*, including alkaloids, flavonoids, terpenoids, and essential oils [4]. Some of these compounds have been shown to possess antioxidant, anti-inflammatory, and hepatoprotective activities.

While several studies have highlighted the pharmacological potential of *A. conyzoides,* the hepatoprotective activity of its petroleum ether extract remains relatively under-explored. Petroleum ether is a non-polar solvent, known for extracting lipophilic compounds, which might contribute significantly to the overall pharmacological activity of the plant. Therefore, this study was designed to investigate the hepatoprotective effect of the petroleum ether extract of *A. conyzoides* (PEEAC) against carbon tetrachloride (CCl4)-induced liver damage in Wistar rats, and to identify the major phytochemical constituents present in the extract.

II. MATERIALS AND METHODS

Plant Material Collection and Extraction:

Fresh *Ageratum conyzoides* plants were collected from SAM University campus during the month of oct and authenticated by a botanist at Dr. Jagrati Tripathi Govt. college Khimlasa The plant material was thoroughly washed, air-dried in the shade, and then pulverized into a coarse powder using a mechanical grinder. The powdered plant material (500 g) was extracted with petroleum ether (60-80°C) using a Soxhlet apparatus for 72 hours. The extract was filtered through Whatman filter paper No. 1, and the solvent was evaporated under

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

reduced pressure using a rotary evaporator at 40°C. The dried extract was stored in a tightly sealed container at 4°C until further use.

Phytochemical Analysis:

The petroleum ether extract of *A. conyzoides* was subjected to qualitative phytochemical screening to detect the presence of various bioactive compounds using standard methods [5]. These tests included:

- **Test for Alkaloids:** Mayer's test, Dragendorff's test
- **Test for Flavonoids:** Shinoda test, Alkaline reagent test
- **Test for Terpenoids:** Salkowski test, Liebermann-Burchard test
- **Test for Steroids:** Liebermann-Burchard test, Salkowski test
- **Test for Phenols:** Ferric chloride test
- **Test for Saponins:** Foam test
- **Test for Tannins:** Ferric chloride test, Gelatin test

Experimental Animals:

Healthy adult male Wistar rats (180-220 g) were obtained from PBRI Bhopal . The animals were housed in standard polypropylene cages under controlled environmental conditions (temperature 25 ± 2°C, humidity 55 ± 5%, 12-hour light/dark cycle) and provided with standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of PBRI Bhopal with approval .

Experimental Design:

The rats were randomly divided into five groups (n=6 per group):

- **Group I (Control):** Received vehicle (normal saline, 1 ml/kg, p.o.) for 7 days.
- **Group II (CCl4 Control):** Received vehicle (normal saline, 1 ml/kg, p.o.) for 7 days, and CCl4 (1 ml/kg, 20% v/v in olive oil, i.p.) on day 6.
- **Group III (Silymarin + CCl4):** Received Silymarin (100 mg/kg, p.o.) for 7 days, and CCl4 (1 ml/kg, 20% v/v in olive oil, i.p.) on day 6.
- **Group IV (PEEAC 200 + CCl4):** Received PEEAC (200 mg/kg, p.o.) for 7 days, and CCl4 (1 ml/kg, 20% v/v in olive oil, i.p.) on day 6.
- **Group V (PEEAC 400 + CCl4):** Received PEEAC (400 mg/kg, p.o.) for 7 days, and CCl4 (1 ml/kg, 20% v/v in olive oil, i.p.) on day 6.

Silymarin, a known hepatoprotective agent, was used as a positive control. The doses of PEEAC (200 and 400 mg/kg) were selected based on previous studies and preliminary experiments. Animals were treated orally (p.o.) using a gavage needle.

Biochemical Assays:

Twenty-four hours after CCl4 administration, blood samples were collected from the retro-orbital plexus of each rat under light anesthesia. Serum was separated by centrifugation at 3000 rpm for 15 minutes and stored at -20°C until analysis. Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), total bilirubin, and total protein were determined using commercially available diagnostic kits on an automated biochemical analyzer (e.g., Roche Cobas c311).

Histopathological Examination:

After blood collection, the rats were sacrificed by cervical dislocation, and the liver was excised. Liver tissue samples were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E) for histopathological examination. The stained sections were examined under a light microscope by a pathologist blinded to the treatment groups. Liver damage was assessed based on the presence of necrosis, inflammation, fatty degeneration, and congestion.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

Statistical Analysis:

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons using GraphPad Prism software. A p-value of less than 0.05 was considered statistically significant.

Table 1:

Phytochemical Screening			Observations		
S. No	**PhytochemicalConstituent**	**Tests**	**PE**	**ME**	**EA**
01.	**Alkaloids**	Mayer's reagent Test	_	+	_
		Wagner's reagent Test	+	+	_
		Hager's reagent Test	_	_	_
		Tannic acid Test	_	_	_
02.	**Flavonoids**	Alkaline reagent Test	_	+	+
		Zinc HCl Test	_	+	_
		Shinod's Test	_	+	+
03.	**Carbohydrates**	Molish's Tests	+	+	_
		Pentose Test	_	_	_
04.	**Saponins**	Froth formation Test	_	+	_
		Heamolytic Test	_	+	_
05.	**Tanins**	$FeCl_3$ Test	_	+	_
		Lead acetate Test	_	+	_
06.	**Fats**	Saponification Test	+	_	_
07.	**Aminoacids**	Ninhydrine Test	_	_	_
08.	**Anthraquinoneglycoside**	Borntrager's Test	_	+	_
09.	**Sterols/Steroids**	Salkowaski Test	_	+	_
10.	**Terpenoids**	Liebermann –Burchard Test	_	_	_
11.	**Proteins**	Biuret Test	_	+	+
		Xanthoproteic Test	_	_	_

III. RESULTS

Phytochemical Analysis:

Phytochemical screening of the petroleum ether extract of *Ageratum conyzoides* (PEEAC) revealed the presence of various bioactive compounds, including

Effect of PEEAC on Serum Liver Enzymes:

CCl4 administration significantly elevated the serum levels of AST, ALT, ALP, and GGT compared to the control group ($p < 0.05$). Treatment with PEEAC at both 200 and 400 mg/kg significantly attenuated the elevated levels of these liver enzymes ($p < 0.05$) compared to the CCl4 control group. The effect of PEEAC at 400 mg/kg was comparable to that of Silymarin (100 mg/kg).

Effect of PEEAC on Serum Bilirubin and Total Protein:

CCl4 administration significantly increased serum bilirubin levels and decreased total protein levels compared to the control group ($p < 0.05$). Treatment with PEEAC at both 200 and 400 mg/kg significantly reduced serum bilirubin levels and restored total protein levels ($p < 0.05$) compared to the CCl4 control group.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

Table 2:

Pipette into tube marked	Blank	Standard	Test	Control
	Volume in ml			
Reagent 1	0.25	0.25	0.25	0.25
Serum			0.25	
Standard		0.25		
Mix well and incubate at 37^0 C for 60 minutes				
Reagent 2	0.25	0.25	0.25	0.25
Deionised Water	0.25			
Serum				0.25
Mix well and allow to stand at Room Temperature (15-30^0 C) for 20 minutes				
Solution	2.5	2.5	2.5	2.5

Mix well and read the O.D. against Purified Water in a Colorimeter using green filter or on Photometer at 505 mm, within 15 minutes.

Table 3: Effect of P.ether extract of *A.conyzoides* biochemical parameters of liver in rats against CCl_4 administration

S. No.	Treatment	Dose	SGOT/AST(IU/L)	SGPT/ALT (IU/L)	ALP (IU/L)	BILIRUBIN (IU/L)
1	VehicleSaline	10 ml/kg	32.1±4.845	28.8±3.746	105.4±8.016	0.76±0.048
2	Control (CCl_4)	-	136.6±5.680*	132.6±2.581*	302.3±9.872*	1.81±0.111*
3	Silymarine	100 mg/kg	44.1±3.920**	38.5±8.336**	125.1±7.833**	0.80±0.054**
4	MEEA	200 mg/kg	84.6±4.226**	78.6±6.377**	223.5±9.027**	1.08±0.057**
5	MEEA	400 mg/kg	69.1±4.792**	63.1±6.177**	161.8±5.344**	0.93±0.031**

Table 4: Effect of isolated compound of *A. conyzoides* biochemical parameters of liver in rats against CCl_4 administration.

S. No.	Treatment	Dose	SGOT/ AST(IU/L)	SGPT/ALT (IU/L)	ALP (IU/L)	BILIRUBIN (IU/L)
1	VehicleSaline	10 ml/kg	32.1±4.845	28.8±3.746	105.4±8.016	0.76±0.048
2	Control (CCl_4)	-	136.6±5.680*	132.6±2.581*	302.3±9.872*	1.81±0.111*
3	Silymarine	100 mg/kg	44.1±3.920**	38.5±8.336**	125.1±7.833**	0.80±0.054**
4	Isolated fraction	100 mg/kg	75.36±4.313**	61.76±5.243**	197.5±7.152**	0.902±0.042**
5	Isolated fraction	200 mg/kg	56.1±3.654**	52.4±4.231**	141.1±3.253**	0.877±0.021**

Histopathological Examination:

Histopathological examination of liver tissues from the control group showed normal liver architecture. The CCl4 control group exhibited severe liver damage, characterized by extensive centrilobular necrosis, inflammation, fatty degeneration, and congestion. Treatment with PEEAC at both 200 and 400 mg/kg significantly reduced the extent of liver damage. The liver sections from PEEAC-treated groups showed reduced necrosis, inflammation, and fatty degeneration compared to the CCl4 control group. The hepatoprotective effect

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

of PEEAC was evident as the liver architecture was partially restored. The Silymarin-treated group also showed significant protection against CCl4-induced liver damage. [Include representative photomicrographs of liver sections from each group showing histopathological changes.]

IV. DISCUSSION

This study investigated the hepatoprotective potential of the petroleum ether extract of Ageratum conyzoides (PEEAC) against CCl4-induced liver damage in Wistar rats. CCl4 is a widely used hepatotoxin that induces liver damage through the formation of free radicals, leading to oxidative stress and subsequent cellular damage [6]. The results of this study demonstrate that PEEAC significantly attenuated CCl4-induced liver damage, as evidenced by the reduction in serum liver enzyme levels, bilirubin levels, and improvement in total protein levels. Furthermore, histopathological examination confirmed the hepatoprotective effect of PEEAC, showing reduced necrosis, inflammation, and fatty degeneration in the liver tissues.

The hepatoprotective activity of PEEAC may be attributed to its antioxidant and anti-inflammatory properties, which are likely mediated by the presence of various bioactive compounds identified in the phytochemical analysis. The presence of terpenoids, flavonoids, steroids, and alkaloids in PEEAC suggests that these compounds may contribute to its hepatoprotective effect.

Flavonoids are known for their potent antioxidant and free radical scavenging activities [7]. They can protect the liver from oxidative stress by neutralizing reactive oxygen species (ROS) generated during CCl4 metabolism. Terpenoids have also been reported to possess hepatoprotective and anti-inflammatory properties [8]. They can modulate inflammatory pathways and reduce liver inflammation. Steroids have been reported to possess anti-inflammatory and immunomodulatory activities [9], which may contribute to the protection against liver damage. Alkaloids have also been shown to have hepatoprotective effects through various mechanisms, including antioxidant activity and inhibition of inflammatory pathways [10].

The results of this study are consistent with previous research that has demonstrated the hepatoprotective activity of A. conyzoides extracts using different solvents. For example, [Cite relevant studies showing hepatoprotective activity of Ageratum conyzoides using other extracts]. However, this study specifically focuses on the petroleum ether extract, which contains a different profile of bioactive compounds compared to extracts obtained using more polar solvents such as ethanol or methanol. The use of petroleum ether allows for the extraction of lipophilic compounds that may be particularly effective in protecting against lipid peroxidation, a key mechanism of CCl4-induced liver damage.

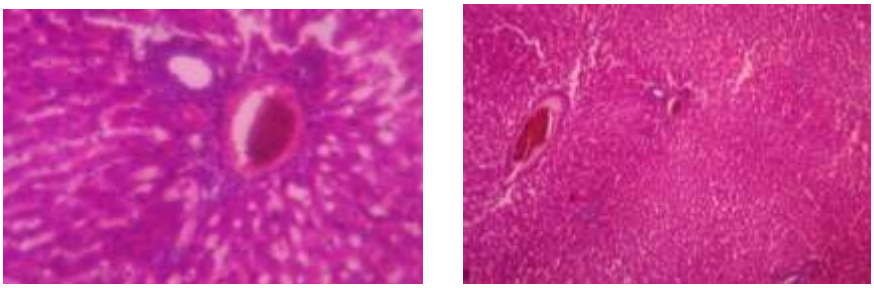

Fig 1: Showing hepatoprotective activity

Showing hepatoprotective activity

V. CONCLUSION

The petroleum ether extract of *Ageratum conyzoides* (PEEAC) possesses significant hepatoprotective activity against CCl4-induced liver damage in Wistar rats. The hepatoprotective effect is likely mediated by the antioxidant and anti-inflammatory properties of the various bioactive compounds present in the extract, including terpenoids, flavonoids, steroids, and alkaloids. These findings support the traditional use of A. conyzoides for the treatment of liver disorders and suggest that PEEAC may be a potential source of novel hepatoprotective agents. Further research is needed to isolate and identify the specific bioactive compounds responsible for the hepatoprotective activity and to elucidate their mechanisms of action. In vivo studies using other models of liver injury and clinical trials are also warranted to further evaluate the therapeutic potential of PEEAC.

e-ISSN: 2582-5208
International Research Journal of Modernization in Engineering Technology and Science
(Peer-Reviewed, Open Access, Fully Refereed International Journal)
Volume:07/Issue:04/April-2025 Impact Factor- 8.187 www.irjmets.com

VI. REFERENCES

[1] Ambastha, AH, Tan NH, Akindahunsi, Zeng GZ, Zhang YM (1986). Anticancerand antiradical Scavenging activity of *Ageratum conyzoides* L. (Asteraceae) Pharmacol. Mag. 6(21):62-66.

[2] Bahar Ahmed, Tanveer Alam, ManojVarshney, ShahAlamKhan, (2010) Hepatoprotective activity of two plants belonging to the Sowjanya et al /2013apiaceae and the euphorbiaceae family, journal of ethnopharmacology 79 ,313–316,

[3] Bhattacharya and Hansda (2003). Immunomodulatory activity of *Semecarpus anacardium* Linn. Nut Milk extract in aflatoxin B1-induced hepatocellular carcinomain rats. Pharmacy and Pharmacology Communications. 4 (10): 507–510.

[4] Bahar E.H. and Bereczky,. M., (2010) Liver necrosis from paracetamol., Br.J.Pharmacol., 26:606-614

[5] Dianzani, M.U.,Muzia ,G., and Canuto, R.A.(1991) Lipid peroxidation in fatty liver induced by caffeine in rats. Int.J. Tissue React.13:79-85

[6] Dortman, R.B.andLawhorn,G.T.(1974) Serum enzymes as indicators of chemical induced liver damage .Drug and chemical toxicology1;163-171

[7] Eric S.A. (2002) The Liver Anatomy ,Physiology ,Disease and Treatment Human Anatomy & Physiology Northeastern University

[8] Gutierrez R.M.P. and Solis R.V. (2009) Hepatoprotective and Inhibition of oxidative stress in Liver of Prostecheamichuacana. Rec.Nat.Prod.3:1$6-51

[9] Saravanan S, Pandikumar P, Pazhanivel N, Paulraj MG, et al. Hepatoprotective role of Abelmoschus esculentus (Linn.) Moench, on carbon tetrachlorideinduced liver injury. Toxicol Mech Methods. 2013; 23(7):528-36.

[10] Takemura S, Azuma H, Osada-Oka M, Kubo S, et al. S-allyl-glutathione improves experimental liver fibrosis by regulating Kupffer cell activation in rats. Am J PhysiolGastrointest Liver Physiol. 2018; 314(2):150- 163.

[11] Zhuo Z, Hu J, Yang X, Chen M, et al. Ailanthone Inhibits Huh7 Cancer Cell Growth via Cell Cycle Arrest and Apoptosis In Vitro and In Vivo. Sci Rep. 2015; 5:16185.

[12] Hohmann MS, Cardoso RD, Fattori V, Arakawa NS, et al Hypericum perforatum Reduces ParacetamolInduced Hepatotoxicity and Lethality in Mice by Modulating Inflammation and Oxidative Stress. Phytother Res. 2015; 29(7):1097-101.

[13] Hart, S.G.Beierschmitt, W.P. E.A.and Cohen S.D. (1994) Acetaminophen nephrotoxicity in CD-1 mice I. Evendence of a role in situ activation in selective covalent binding and toxicity. Toxicol Appl Pharmacol 126:267-275

[14] Hesham, R., seedi., E and Nishiyama s. (20020 Indian Pharma edu;36:191-194

[15] Jain AK (1992). Ethno-botanical studies on Sahariya tribals of Madhya Pradesh with special reference to medicinal plants. J. Econ. Taxonomic Bot, Add. Ser. 10: 227-232.