Bulletin of Science and Technology

Content Available at www.lapinpress.com ISSN (O): XXXX-XXXX
(An online peer reviewed Referred Journal)

Review Article

OPEN ACCESS

EVALUATION OF HEPATOPROTECTIVE ACTIVITY OF AERIAL EXTRACTS OF *TRAPA NATANS*AGAINST CCL_4 INDUCED HEPATOTOXICITY

BOREDDY HIMAJA[1]*, M.SRINIVASA MURTHY[2], K.SARITHA[3], ASRA TAYABBA[4], B.KAVYA[5], B.VIGNESHWAR[6]

[1]*Assistant Professor, Department of Pharmacology, Vignan Institute of Pharmaceutical Sciences, Near Ramoji film city, Yadadri Bhuvanagiri Dist, Telangana.*
[2]*Professor & Principal, Department of Pharmaceutical Chemistry, Vignan Institute of Pharmaceutical Sciences, Near Ramoji film city, Yadadri Bhuvanagiri Dist, Telangana.*
[3]*Associate Professor, HOD, Department of Pharmacognosy, Vignan Institute of Pharmaceutical Sciences, Near Ramojifilmcity, Yadadri Bhuvanagiri Dist, Telangana.*
[4]*Vignan Institute of Pharmaceutical Sciences, Near Ramojifilmcity, YadadriBhuvanagiriDist, Telangana.*
[5]*Vignan Institute of Pharmaceutical Sciences, Near Ramojifilmcity, YadadriBhuvanagiriDist, Telangana.*
[6]*Vignan Institute of Pharmaceutical Sciences, Near Ramojifilmcity, YadadriBhuvanagiriDist, Telangana.*
[7]*Vignan Institute of Pharmaceutical Sciences, Near Ramojifilmcity, YadadriBhuvanagiriDist, Telangana.*

***Corresponding Author**
Boreddy Himaja

Article History: Received: 04 April 2026, Revised: 26 Apr 2026, Accepted: 10 May 2026

Abstract: This study aimed to investigate the hepatoprotective activity of methanolic extracts of *Trapa natans against* all the chemical induced hepatotoxicity. The hepatoprotective activity was evaluated by using Carbon tetrachloride (CCl_4) induced toxicity models in rats. Silymarin was used as a standard drug. This chemical is well-known hepatotoxin, when exposure to this chemical is known to induce oxidative stress and causes liver injury by the formation of free radicals. Animals were pre-treated with the methanolic extracts of *Trapa natans for* one week and then challenged with CCl_4 (1.5 ml/kg) in olive oil (1:1, v/v) on 7thday . Serum marker enzymes (ALP, AST, ALT and Total bilirubin) were estimated in all the study groups. Alteration in the levels of biochemical markers of hepatic damage like AST, ALT, ALP and Total bilirubin were tested in both toxic-treated and extract-treated groups. The results of the present study indicates that the plant extract showed a significant decrease in the serum levels of ALP, AST, ALT and Total bilirubin. These biochemical observations were supported by a histopathological study of liver sections. This study confirmed the hepatoprotective property of this plant as this was used in traditional medicine.

Keywords: *CCl_4, Hepatotoxicity, Hepatoprotective activity, Biochemical markers.*

This article is licensed under a Creative Commons Attribution-Non-commercial 4.0 International License.
Copyright © 2026 Author(s) retains the copyright of this article.

INTRODUCTION

The liver is one of the largest and most vital organs in the human body, playing a central role in maintaining metabolic homeostasis [1]. It performs numerous physiological functions, including the metabolism of carbohydrates, proteins, and lipids; detoxification of endogenous and exogenous substances; synthesis of plasma proteins and clotting factors; storage of vitamins and minerals; and production of bile necessary for digestion and absorption of fats. Owing to its strategic position in xenobiotic metabolism, the liver is continuously exposed to a wide range of toxic chemicals, drugs, environmental pollutants, and infectious agents, making it highly susceptible to injury.

Liver diseases represent a major global health concern, contributing significantly to morbidity and mortality worldwide30. Hepatotoxicity can result from excessive alcohol consumption, viral infections, autoimmune disorders, exposure to industrial chemicals, environmental toxins, and the prolonged use of certain medications. Despite advances in modern medicine, effective therapeutic options for the prevention and treatment of liver diseases remain limited. Most currently available drugs provide only symptomatic relief and are often associated with undesirable adverse effects [2, 3]. Therefore, there is a growing interest in identifying safe, effective, and affordable hepatoprotective agents from natural sources.

Medicinal plants have been used for centuries in traditional systems of medicine for the management of liver disorders. They are rich in biologically active phytochemicals such as flavonoids, phenolic acids, tannins, alkaloids, terpenoids, and saponins, which possess antioxidant, anti-inflammatory, and cytoprotective properties [4]. These phytoconstituents help neutralize reactive oxygen species, inhibit lipid peroxidation, stabilize cellular membranes, and enhance the regeneration

of damaged hepatocytes5. Consequently, plant-derived hepatoprotective agents continue to attract considerable scientific attention. *Trapa natans*L., commonly known as water chestnut, is an aquatic annual plant belonging to the family Lythraceae. It is widely distributed in freshwater bodies across Asia and Europe and has been traditionally utilized for its nutritional and medicinal value6. Various parts of the plant, including its fruits, leaves, and stems, contain a wide range of phytochemicals such as flavonoids, phenolic compounds, tannins, triterpenoids, and glycosides [7]. Previous pharmacological investigations have demonstrated that *Trapa natans*exhibits antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and anticancer activities. The strong antioxidant potential of its phytoconstituents suggests that the plant may also possess significant hepatoprotective activity by protecting hepatic tissues against oxidative damage [8,9]. Carbon tetrachloride (CCl_4) is one of the most extensively employed experimental hepatotoxins for evaluating hepatoprotective agents. Following metabolic activation by the cytochrome P450 enzyme system in hepatocytes, CCl_4 is converted into highly reactive trichloromethyl ($\bullet CCl_3$) and trichloromethyl peroxy ($\bullet OOCCl_3$) free radicals [10]. These reactive intermediates initiate lipid peroxidation of cellular membranes, resulting in oxidative stress, mitochondrial dysfunction, inflammation, hepatocellular necrosis, and leakage of intracellular enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) into the bloodstream. Due to its reproducibility and close resemblance to human liver injury, the CCl_4-induced hepatotoxicity model remains a standard experimental model for screening hepatoprotective compounds [11,12]. The leaves of *Trapa natans*are particularly rich in polyphenols and flavonoids, which are recognized for their potent free radical scavenging and antioxidant activities13. These bioactive constituents may effectively counteract oxidative stress, reduce inflammatory responses, preserve the structural integrity of hepatocytes, and promote liver regeneration following toxic injury [14,15].

MATERIALS AND METHODS

Plant Material and Extraction:

Fresh leaves of *Trapa natans*were collected from local freshwater bodies and authenticated by a qualified taxonomist. A voucher specimen was deposited in the Department of Pharmacognosy for future reference. The collected leaves were thoroughly washed, shade-dried, and powdered using a mechanical grinder. The powdered material was extracted with ethanol using a Soxhlet apparatus16. The extract was concentrated under reduced pressure using a rotary vacuum evaporator and stored in an airtight container at 4°C until further use [17].

Preliminary Phytochemical Screening:

The ethanolic leaf extract of *Trapa natans*was subjected to preliminary qualitative phytochemical screening using standard methods to determine the presence of alkaloids, flavonoids, tannins, phenolic compounds, saponins, glycosides, steroids, terpenoids, carbohydrates, proteins, amino acids, and fixed oils [18-20].

Chemicals

Carbon tetrachloride (CCl_4), silymarin, olive oil, and commercially available biochemical diagnostic kits for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin were of analytical grade and obtained from standard commercial suppliers.

Experimental Animals

Healthy adult Swiss albino rats of either sex, weighing 150–200 g, were obtained from a CPCSEA-approved animal facility [21]. The animals were housed under standard laboratory conditions (22 ± 2°C, 55 ± 5% relative humidity, and a 12 h light/12 h dark cycle) with free access to standard pellet diet and water. They were acclimatized for one week prior to the experiment [22].

Acute Oral Toxicity Study

Acute oral toxicity of the ethanolic leaf extract was evaluated according to OECD Guideline 423. The extract was administered orally at a limit dose of 2000 mg/kg body weight23,34. Animals were observed continuously during the first 4 h and periodically for 14 days for mortality and signs of toxicity. Based on the findings, doses of 200 and 400 mg/kg body weight were selected for the hepatoprotective study [24].

Experimental Design

Thirty Swiss albino rats were randomly divided into five groups (n = 6). Group I served as the normal control and received vehicle only25. Group II served as the toxic control and received CCl_4. Group III received silymarin (100 mg/kg, p.o.) along with CCl_4. Groups IV and V received the ethanolic leaf extract of *Trapa natans*at doses of 200 and 400 mg/kg body weight, respectively, along with CCl_4. The extract and silymarin were administered orally once daily for 14 consecutive days. Hepatotoxicity was induced by intraperitoneal administration of CCl_4 (1 mL/kg, 1:1 v/v in olive oil) according to the experimental protocol [26-28].

Biochemical Analysis

Twenty-four hours after the final treatment, blood samples were collected, and serum was separated by centrifugation. Serum ALT, AST, ALP, total bilirubin, total protein, and albumin levels were estimated using commercially available diagnostic kits according to the manufacturer's instructions [29,33].

Histopathological Examination

Liver tissues were excised immediately after sacrifice, washed with normal saline, fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, sectioned at 4–5 μm thickness, stained with hematoxylin and eosin, and examined under a light microscope for histopathological alterations [30-32].

Statistical Analysis

Data were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using GraphPad Prism software. A value of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

1. Preliminary Phytochemical Screening

The qualitative phytochemical screening of the methanolic leaf extract of *Trapa natans*revealed the presence of several bioactive phytoconstituents including flavonoids, phenolic compounds, tannins, saponins, alkaloids, glycosides and terpenoids, while steroids were present in trace amounts. These phytochemicals are known to possess antioxidant and free radical scavenging activities, which may contribute to the hepatoprotective potential of the extract.

Table 01: Phytochemical screening

S.No	Constituents	Test	Methanolic extract
1.	Alkaloids	A) Mayer'sreagent B) Wagner'sreagent C) Dragendroff'sreagent D) Hagner'sreagent	Present
2	Carbohydrates	a) Molisch'sreagent b) Benedictsreagent c) Fehling'ssolution	Absent
3	Glycosides	a) Modifiedborntrager'stest b) Legaltest	Present
4	Phytosterol	a) Salkowski'stest b) LibermannBurchard's test	absent
5	saponins	a) Frothtest b) Foamtest	Present
6	Tannins	Gelatintest	Present
7	Proteins	a) Xanthoproteintest b) Ninhydrintest	Present
8	Flavonoids	a) Alkalinereagenttest b) Leadacetatetest	Present

2. Effect on Body Weight

Animals treated with CCl_4 showed a significant reduction in body weight compared with the normal control group. Treatment with *Trapa natans*leaf extract at both doses significantly restored body weight. The higher dose exhibited greater improvement, comparable to the standard drug.

Discussion

Body weight loss observed in CCl_4-treated rats may be attributed to impaired liver function and reduced protein synthesis. Restoration of body weight following treatment indicates improvement in hepatic metabolism and overall health.

3. Effect on Liver Function Biomarkers

CCl_4 administration produced a significant elevation in serum ALT, AST, ALP, and total bilirubin levels, while total protein and albumin levels were markedly decreased. Treatment with *Trapa natans*leaf extract significantly decreased serum ALT, AST, ALP, and bilirubin levels in a dose-dependent manner and restored protein and albumin concentrations toward normal values.

Table 02: Liver Function Biomarkers

Groups	ALT	AST	ALP	Bilirubin
Control	48.16±0.55	118.3±0.805	427.5±0.697	0.345±0.006
Toxiccontrol	320± 0.663	617.33±0.902	754.66±0.652	0.871±0.005
Silymarin	137± 0.781***	439.5±0.697***	564.3±0.608***	0.545±0.006
MELI	240.5±0.39***	422.5±0.697***	612.16±0.641***	0.865±0.003

MELI	201±0.744***	409±0.881***	578.66±0.693***	0.885±0.006

Each value is the mean ± SEM for 6 mice, *P<0.5, **P<0.01, ***P<0.001. Compared with control, data were analysed by using one-way ANOVA followed by Dunnet's test, standard Silymarin (5 ml/kg; p.o) MELI- Methanolic extract of *Trapa natans*, dose (250mg/kg of B.W) MELI- Methanolic extract of *Trapa natans*(500mg/kg of B.W),

DISCUSSION

Elevation of serum liver enzymes is a characteristic indicator of hepatocellular damage caused by leakage from damaged hepatocytes. The reduction of these enzymes after treatment suggests stabilization of hepatocyte membranes and regeneration of liver cells. Improvement in serum protein and albumin further confirms restoration of the liver's synthetic function.

4. Antioxidant Parameters

CCl_4 intoxication significantly increased malondialdehyde (MDA) levels while decreasing superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) activities.

Administration of *Trapa natans*extract significantly reduced lipid peroxidation and restored endogenous antioxidant enzyme levels.

Discussion

Carbon tetrachloride metabolism generates trichloromethyl radicals that initiate lipid peroxidation, leading to oxidative stress. The antioxidant phytoconstituents of Trapa natans, particularly flavonoids and phenolics, effectively neutralize reactive oxygen species, thereby protecting hepatocytes from oxidative injury.

5. Histopathological Examination

Histological examination of normal liver sections showed intact hepatic architecture with normal hepatocytes, central vein, and hepatic sinusoids.

Liver sections from the CCl_4-treated group exhibited severe hepatocellular degeneration, fatty changes, inflammatory cell infiltration, sinusoidal congestion, and centrilobular necrosis.

Treatment with *Trapa natans*leaf extract markedly improved hepatic architecture. The low-dose group showed moderate recovery, whereas the high-dose group demonstrated nearly normal hepatic architecture with minimal inflammatory changes, closely resembling the standard treatment group.

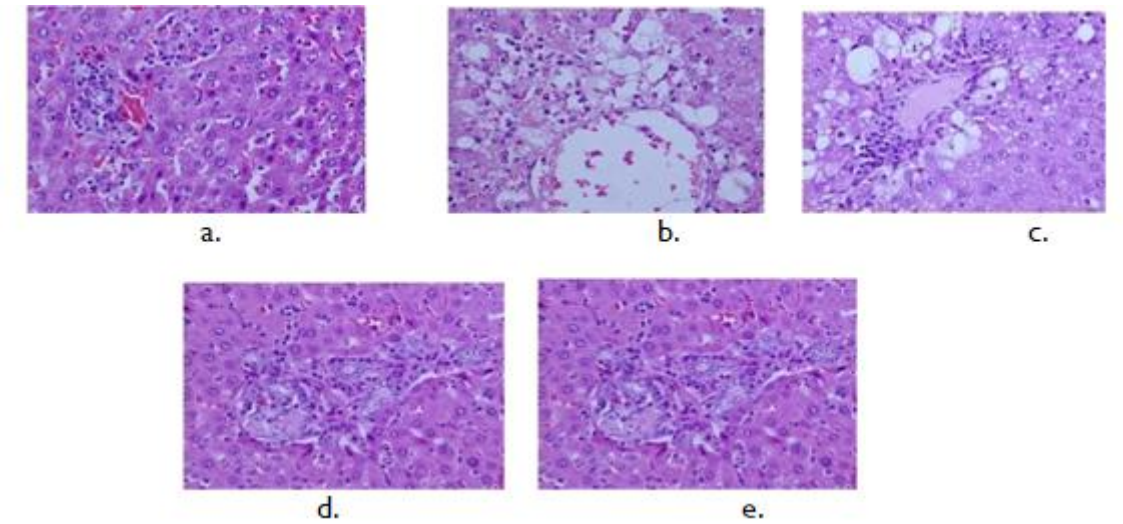

Figure 01: (a)Sectionofthelivertissueofcontrolanimal;(b)Sectionoflivertissueofanimaltreated with CCl4; (c) Section of liver tissue of methanolic extract of *Trapa natansI*, treated animal;(d)Sectionofthelivertissueofmethanolicextractof*Trapa natansII*,;(e)Section of the liver tissue of Silymarin treated animal.

Discussion

Histopathological findings strongly support the biochemical results. The hepatoprotective activity of *Trapa natans*appears to result from its antioxidant and membrane-stabilizing effects, preventing hepatocyte necrosis and promoting liver regeneration.

OVERALL DISCUSSION

The present study demonstrates that the methanolic leaf extract of *Trapa natans*possesses significant hepatoprotective activity against CCl_4-induced liver injury in Swiss albino rats. Hepatoprotection was evidenced by normalization of liver

function biomarkers, improvement in antioxidant defense systems, restoration of body weight, and preservation of normal hepatic histology.

The hepatoprotective effect may be attributed to the synergistic action of flavonoids, phenolic compounds, tannins, and other phytoconstituents that scavenge free radicals, inhibit lipid peroxidation, stabilize cellular membranes, and promote regeneration of damaged liver tissue.

Overall, the higher dose of *Trapa natans*leaf extract demonstrated superior hepatoprotective activity and produced effects comparable to those of the standard hepatoprotective drug, suggesting that *Trapa natans*may serve as a promising natural therapeutic agent for the management of liver disorders.

The present study demonstrated that the methanolic leaf extract of *Trapa natans*possesses significant hepatoprotective activity against carbon tetrachloride (CCl_4)-induced hepatotoxicity in Swiss albino rats. Phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides, and terpenoids, which are known for their antioxidant and hepatoprotective properties.

Administration of the leaf extract significantly reduced the elevated serum hepatic biomarkers (ALT, AST, ALP, and total bilirubin) and restored serum total protein and albumin levels toward normal. The extract also improved the antioxidant defense system by reducing lipid peroxidation and enhancing the activities of endogenous antioxidant enzymes. Histopathological examination further confirmed protection against CCl_4-induced hepatic damage, with marked restoration of normal liver architecture, particularly at the higher dose.

The hepatoprotective effect of *Trapa natans*is likely mediated through its antioxidant, free radical scavenging, membrane-stabilizing, and cytoprotective mechanisms. These findings suggest that *Trapa natans*leaf extract has considerable potential as a natural hepatoprotective agent and may be useful in the prevention and management of liver disorders associated with oxidative stress.

Further studies are recommended to isolate and characterize the active phytoconstituents, elucidate their molecular mechanisms of action, evaluate long-term safety, and conduct clinical studies to establish the therapeutic potential of *Trapa natans*in human liver diseases.

CONCLUSION

The present study demonstrates that the methanolic leaf extract of *Trapa natans* possesses significant hepatoprotective activity against carbon tetrachloride (CCl_4)-induced hepatic injury in Swiss albino rats. The extract effectively attenuated the CCl_4-induced elevation of serum ALT, AST, ALP, and total bilirubin levels while improving total protein and albumin concentrations. It also enhanced the endogenous antioxidant defense system by reducing lipid peroxidation and restoring antioxidant enzyme activities.

The histopathological findings further supported the biochemical results, showing marked protection and restoration of hepatic architecture following treatment with the extract. The hepatoprotective activity may be attributed to the presence of flavonoids, phenolic compounds, tannins, alkaloids, saponins, glycosides, and terpenoids, which may exert antioxidant, free radical-scavenging, membrane-stabilizing, and cytoprotective effects.

Among the tested doses, the higher dose exhibited greater hepatoprotective activity and produced effects approaching those of the standard drug, silymarin. Thus, *Trapa natans* leaves may represent a promising natural source of hepatoprotective agents. However, further investigations are required to identify the active constituents, clarify their molecular mechanisms, establish long-term safety, and validate these findings through further preclinical and clinical studies.

FUNDING

Nil

ACKNOWLEDGEMENT

Not Declared.

CONFLICT OF INTEREST

Nil

INFORMED CONSENT

Not applicable

ETHICAL STATEMENT

Not Applicable.

REFERENCES

1. Recknagel RO, Glende EA Jr, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. PharmacolTher. 1989;43(1):139-154.

2. Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury. Hepatology. 2012;55(3):965-967.
3. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003;349(5):474-485.
4. Zimmerman HJ. Hepatotoxicity: The Adverse Effects of Drugs and Other Chemicals on the Liver. 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 1999.
5. Friedman SL. Liver fibrosis—from bench to bedside. J Hepatol. 2003;38(Suppl 1):S38-S53.
6. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351-358.
7. Aebi H. Catalase in vitro. Methods Enzymol. 1984;105:121-126.
8. Ellman GL. Tissue sulfhydryl groups. Arch BiochemBiophys. 1959;82(1):70-77.
9. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J BiochemBiophys. 1984;21:130-132.
10. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 7th ed. Philadelphia: Churchill Livingstone Elsevier; 2013.
11. OECD. OECD Guideline for the Testing of Chemicals 423: Acute Oral Toxicity–Acute Toxic Class Method. Paris: OECD; 2001.
12. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd ed. London: Chapman & Hall; 1998.
13. Trease GE, Evans WC. Trease and Evans Pharmacognosy. 16th ed. London: Saunders Elsevier; 2009.
14. Kokate CK. Practical Pharmacognosy. 5th ed. Pune: NiraliPrakashan; 2008.
15. Evans WC. Trease and Evans Pharmacognosy. 15th ed. London: Saunders; 2002.
16. Hussain T, Gupta RK, Sweety K, Khan MS, Hussain MS, Arif M, et al. Hepatoprotective evaluation of *Trapa natans*against antitubercular drug-induced hepatotoxicity in rats. Pharmacogn Mag. 2018;14(55):170-177.
17. Kang WY, Li YY, Gu XZ, Huang X. Hepatoprotective activity of Trapa acornis shell extracts against carbon tetrachloride-induced liver injury in rats. Afr J Pharm Pharmacol. 2012;6:2856-2861.
18. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. ScientificWorldJournal. 2013;2013:162750.
19. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 5th ed. Oxford: Oxford University Press; 2015.
20. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84.
21. Sies H. Oxidative stress: Oxidants and antioxidants. Exp Physiol. 1997;82(2):291-295.
22. Poli G. Pathogenesis of liver fibrosis: Role of oxidative stress. Mol Aspects Med. 2000;21(3):49-98.
23. Gupta M, Mazumder UK, Kumar RS, Kumar TS. Studies on hepatoprotective activity of medicinal plants. Indian J Exp Biol. 2004;42:123-127.
24. Mukherjee PK. Quality Control of Herbal Drugs. 2nd ed. New Delhi: Business Horizons; 2007.
25. Kirtikar KR, Basu BD. Indian Medicinal Plants. 2nd ed. Dehradun: International Book Distributors; 1999.
26. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 49th ed. Pune: NiraliPrakashan; 2014.
27. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 3rd ed. Ibadan: Spectrum Books; 2008.
28. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd ed. Berlin: Springer; 1996.
29. World Health Organization. WHO Guidelines on Good Agricultural and Collection Practices (GACP) for Medicinal Plants. Geneva: WHO; 2003.
30. World Health Organization. WHO Traditional Medicine Strategy 2014–2023. Geneva: World Health Organization; 2013.
31. Kodithala S, Uppuluru AK, Miya S, Kumar CS, Mansoor M, Sandya B. Phytochemical landscape of Cordia latifolia Roxb: mapping bioactivity and molecular structures. World Journal of Pharmacy and Pharmaceutical Sciences. 2026;15(5):429-435.
32. Murthy MS, Kodithala S, Mansoor M, Kumar CS, Uppuluru AK, Miya S. Computational docking study of phytochemicals isolated from Barleriaalbostellata C.B Clarke. International Journal of Pharmaceutical Sciences. 2026;4(4):1970-1979.
33. Miya S, Murthy MS, Kodithala S, Mansoor M, Kumar CS, Uppuluru AK. Deciphering the shield: Callisia repens leaf extract attenuates oxidative stress and hepatic injury in experimental rat models. World Journal of Pharmaceutical Research. 2026;15(9).
34. Kumar CS, Kodithala S, Uppuluru AK, Miya S, Mansoor M, Sandya B. Bioactive profiling and therapeutic potential of Eriocapitella tomentosa: a hunt for novel antimicrobial leads. European Journal of Pharmaceutical and Medical Research. 2026;13(5).
35. Mansoor M, Kodithala S, Uppuluru AK, Miya S, Kumar CS, Sandya B. Navigating the Pain Matrix: A Comparative Study on the Systemic Analgesic Potency of Arnica montana in Wistar Rats, International Journal of Advanced Scientific and Technical Research. April 2026;16(2).