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# *PHARMACOGNOSTIC, IN VITRO ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF LEAVES OF GARDEN CROTON, CODIAEUM VARIEGATUM (EUPHORBIACEAE)*

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*Antioxidant, Garden croton, Phenolic compound, oxidative stress, hepatoprotective activity.*

***ABSTRACT***
**Background:** Commonly referred to as garden croton, *Codiaeum variegatum* is a tropical decorative plant that is valued for both its therapeutic properties and its colourful foliage. The present study investigates the antioxidant and hepatoprotective properties of garden croton. **Methodology:** The phytochemical screening was conducted to determine the amount of polyphenols present in the sample. The Total Phenolic and flavonoid content was performed by the Folin and Aluminium chloride method, respectively. Furthermore, the samples were evaluated using various antioxidant methods. Hepatoprotective activities of the crude extract of the plant were carried out based on paracetamol-induced liver damage in mice. Serum biomarkers (AST, ALT, ALP, and hepatotoxicity index) were assessed to determine the effect. Histopathological examination was also performed on all groups of mice to further confirm the findings. **Result and Discussion:** The different methods revealed that the antioxidant potential increased with higher concentrations of polyphenols in the sample. The $IC_{50}$ values ranged from 99.28 to 115.22 µg/mL with three different methods. Pre-treatment of the mice with the crude extract of Croton significantly reduced ALP ($p < 0.05$), ALT ($p < 0.05$), and AST ($p < 0.05$) levels at all administered doses compared to the toxic group. **Conclusion:** Croton leaves may serve as a natural source of novel compounds with hepatoprotective properties. These results suggest that Ornamental plants also contain a higher concentration of bioactive compounds capable of neutralizing free radicals and show hepatoprotective activity at very low doses. However, further research is recommended to isolate and characterize the specific phytoconstituents responsible for the activity and to elucidate their mechanisms of action in greater detail.

## *INTRODUCTION*

Ornamental plants are valuable as they can attract clients. Consequently, ornamentals or cultivars must conform to the specified aesthetic standards. The primary goals of ornamental plant breeding are to enhance growth habit, disease resistance, nutrient absorption, varietal characteristics, novel

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colouration, and reddish pink. The primary goals of ornamental plant breeding are to enhance growth habit, disease resistance, nutrient absorption, varietal characteristics, novel colouration, morphology, dimensions, flower quantity, floral longevity, and recurrent blooming [1] [table 1]. Croton plants have traditionally been utilised to treat various ailments, including ulcers, pain, diarrhoea, constipation, external wounds, inflammation, fever, intestinal worms, diabetes, cancer & hypercholesterolemia [2]. Besides serving as a tonic from its leaves and a purgative from its fruits, seeds, and flowers, the plant *Codiaeum variegatum* is utilised to address flatworm infestations and dysmenorrhea. The roots are used for treating diarrhoea, while the bark is employed for managing dyspepsia [3]. The methanolic extract of garden croton predominantly contains alpha-amyrin, beta-sitosterol, rutin, ellagic acid, p-coumaric acid, and epicatechin. The methanolic extract of garden croton contains five additional significant flavonoids: apigenin, vitexin, vice-2, orientin, and isovitexin, alongside two principal polyphenols, p-coumaric acid and caffeic acid, as indicated by further research [4]. The principal components of Croton were determined to be oxygenated sesquiterpenes, namely (Z)-farnesene (20%), caryophyllene oxide (58%), and sesquiterpene hydrocarbons (33%) [5]. Extracts from *C. variegatum* exhibit features analogous to studies associating antibacterial, antiviral, antioxidant, and anticancer effects with many categories of bioactive metabolites, such as triterpenes, quassinoids, inonoids, flavonoids, alkaloids, and sesquiterpenes [6]. This manuscript aims to comprehensively investigate the macroscopic and microscopic features, chemical constituents, total phenolic and total flavonoid content, antioxidant potential, and hepatoprotective activity of *C. variegatum*.

## *MATERIAL AND METHOD*

### Plant Identification

Leaves of *C. variegatum* were procured from the field area of Varanasi, Uttar Pradesh (India) in the month of February-March in the year 2024. The leaves were authenticated by the Department of Botany, Banaras Hindu University, with reference no. Euphorbia.2023/01 for further study.

**Organoleptic Evaluation:** The organoleptic study of the leaf, including color, shape, size, odor, taste, and distinctive features, was identified and analyzed according to the standard procedures outlined in the Ayurvedic Pharmacopoeia of India [14, 15].

**Table 1: Bioactivity data of other ornamental plants**

| Plant species | Part of the plant | Type of extract | $IC_{50}$ value | Model Used | Dose (mg/kg) | Effect on biochemical markers | Ref. |
|---|---|---|---|---|---|---|---|
| ***Croton macrostachyus*** | Bark | Ethanol extract | 68.3 ± 2.5 | $CCl_4$-induced liver injury | 200 | AST, ALT; mild antioxidant response | 7 |
| ***Adansonia digitata*** | Fruit pulp | Aqueous Extract | 35.0 ± 1.7 | Paracetamol-induced hepatic injury in mice | 250 | Strong antioxidant activity; normalizes liver enzyme levels | 8 |
| ***Lantana camara*** | Leaves | Methanolic Extract | 49.6 ± 0.9 | $CCl_4$-induced liver damage in rats | 150 | hepatoprotection and lipid peroxidation reduction | 9 |
| ***Croton tiglium*** | Seeds | Aqueous extract | 65.4 ± 2.8 | $CCl_4$-induced liver damage in rats | 220 | AST, ALT; mild antioxidant response | 10 |
| ***Croton bonplandianus*** | Whole plant | Ethanolic extract | 72.5 ± 2.1 | Paracetamol-induced hepatotoxicity in rats | 300 | ↓ ALT, ALP, TB; moderate antioxidant response | 11 |
| ***Dypsis lutescens*** | Leaves | Ethanolic Extract | 67.7±1.4 | D-galactosamine induced | 225 | Strong antioxidant activity; normalizes liver enzyme levels | 12 |
| ***Nyctanthes arbor-tristis*** | leaves | Ethanolic Extract | - | $CCl_4$-induced liver damage in rats | 500 | ↓ SGPT, SGOT, and ALP | 13 |

**Microscopical Evaluation:** A transverse section through the midrib was prepared & stained with safranin to highlight various anatomical features. The stained section was then observed under a binocular microscope at magnifications of 100X & 250X.

**Physical Evaluation:** The physicochemical evaluation was conducted according to the guidelines outlined by the WHO (1992), assessing parameters such as extractive values, moisture content, ash content, and foreign matter.

**Phytochemical Evaluation**

In accordance with the Ayurvedic Pharmacopoeia of India, a preliminary phytochemical evaluation of extracts was conducted to identify the presence of phytoconstituents [16].

**Preparation of Extract using Different Solvents**

To remove any clinging contaminants, the fresh leaves were cleaned and dried in the shade. After that, dried leaves were ground into powder using a grinder. The powdered substance passed through a 40-mesh screen. The powdered material was defatted with Petroleum ether (60–80°) using a Soxhlet apparatus. After defatting, the powder is extracted using a variety of solvents, including benzene, chloroform, ethyl acetate, and ethanol. To create the aqueous extract, the leftover residue was further extracted with chloroform-water (1:99). The extracted materials were then concentrated on a water bath and subsequently dried by evaporation. The % yield is calculated with respect to the air-dried weight of the crude drug. The colour and consistency of the extracts were recorded [17].

**Evaluation of Total Phenolic Content**

5g of ethanolic extract was dissolved in 50ml of ethanol in a beaker, which was then transferred to a 100 mL volumetric flask and made up to the mark. 1 mL of this solution was transferred into a 10 mL of volumetric flask containing 2 mL of Folin-Ciocalteu reagent. Add 4 mL of 7.5%w/v sodium carbonate to the mixture. The mixture was then thoroughly mixed and allowed to stand in a dark place for thirty minutes. The absorbance of the resultant sample was estimated by using UV at 765 nm [18, 19]. The TPC of the test sample was evaluated using the linear equation obtained from the standard curve of GAE and expressed as mg of GAE equivalents per extract.

$$\boldsymbol{Y = 0.0025x + 0.0719}; \ R^2 = 0.9974$$

***Where, x= amount of GAE (µg); y= absorbance***

**Evaluation of Total Flavonoid Content**

Initially, 5 mL of ethanolic extract was diluted with ethanol to get a final volume of 100 mL. 1 mL of the stock solution was taken in a 10 mL volumetric flask containing 1 mL of a 10% w/v aluminium chloride solution and 2 mL of a 5% w/v sodium nitrite solution in methanol. To the solution, 2 mL of a 4% w/v sodium hydroxide solution was added, and the final volume was adjusted with water. The absorbance of the mixture was quantified at 415 nm using UV [20, 21]. The TFC in the test solution was evaluated using the regression line of the Quercetin standard curve and represented as mg Quercetin equivalent per extract.

$$\boldsymbol{Y = 0.0038x + 0.0862}; \ R^2 = 0.9957$$

***Where, y = absorbance; x = amount of Quercetin (µg)***

***IN-VITRO* ANTIOXIDANT ACTIVITY**

**DPPH Scavenging Assay**

1 mL of the extract was diluted in methanol at different concentrations (50 to 250 µg/mL); thereafter, 1 mL of a mM DPPH solution in methanol was added to the same. The resultant mixture was thoroughly agitated and incubated in a dark place for approximately 30 minutes. The absorbance was evaluated at 517 nm with a UV-Vis spectrophotometer. DPPH solution was used as the control. The % RSA was determined using the resulting formula [22].

$$\%\ \boldsymbol{of\ Antioxidant\ activity} = \frac{\boldsymbol{Absorbance_{control} - Absorbance_{sample}}}{\boldsymbol{Absorbance_{control}}} \times \mathbf{100}$$

Different concentrations of extract were plotted against %RSA, and the trend line equation was used to calculate $IC_{50}$ concentrations for each sample.

**Nitric Oxide Scavenging Assay**

3 mL of the mixture, which included 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate-buffered saline, and 0.5 mL of the standard (ascorbic acid) or extracts (500–1000 µg/mL), was incubated for 150 minutes at room temperature. Later, 0.5 mL of the mixture was added to 1 mL of 0.33% sulfanilic acid reagent, which was prepared in 20% glacial acetic acid. To this, 1 mL of naphthyl ethylenediamine dihydrochloride was added, the solution was mixed vigorously, and it was then permitted to stand at 25°C for 30 minutes [23]. The nitrite concentration at 546 nm was assessed and established utilising the control absorbance of the standard nitrite solution. Ascorbic acid served as the standard, whereas the buffer solution was taken as the blank [24]. The inhibition % was determined using the subsequent formula.

$$\%\ \boldsymbol{of\ Antioxidant\ activity} = \frac{\boldsymbol{Absorbance_{control} - Absorbance_{sample}}}{\boldsymbol{Absorbance_{control}}} \times \mathbf{100}$$

**Hydrogen Peroxide Scavenging Assay**

A 100 mM hydrogen peroxide solution was added to phosphate buffer (pH 7.4). Two milliliters of the $H_2O_2$ solution were mixed with different concentrations of plant extracts (100–1000

µg/mL). After this, the mixture was allowed to stand in a dark place for 10 minutes, and then the absorbance value was recorded at 230 nm against a blank containing only phosphate buffer. The absorbance of the hydrogen peroxide solution served as a control [25]. The percentage inhibitory activity was calculated using the formula for the % antioxidant activity.

$$\%\ \boldsymbol{of\ Antioxidant\ activity} = \frac{\boldsymbol{Absorbance_{control} - Absorbance_{sample}}}{\boldsymbol{Absorbance_{control}}} \times \mathbf{100}$$

**Assessment of Hepatoprotective Activity**

The handling of the animals complied with the guidelines set fo rth by the animal ethics committee for the care and use of resea rch animals. This investigation was approved ethically by the IMS Department of Pharmacology's research ethics committee (No. Dean/2025/IAEC/7899).

**Experimental Model**

The hepatoprotective activity was assessed using a paracetamol-induced hepatic injury model with certain modifications to the procedure [26, 27]. The animals were categorized into five distinct groups, each consisting of six individuals. The study duration for the animals was 14 days. Before administering the medication, the animals were housed for seven days to allow for acclimatization. During this acclimation phase, the animals were provided with ad libitum access to water and a regular feeding schedule.

The Group I was treated as a normal control group and administered 1% CMC (10 mL/kg, p.o.) daily for seven days. Group II was treated with paracetamol dissolved in 1% CMC (250 mg/kg, p.o) daily for seven days. Group III served as the standard and was treated with Silymarin dissolved in 1% CMC (100 mg/kg/day, p.o.) for seven days. Groups IV and V were treated with the ethanol extract of *C. variegatum* in 1% CMC at 200mg/kg and 400mg/kg, p.o., for seven days. Group III–V animals were intoxicated with paracetamol (250 mg/kg, p.o) after the administration of Silymarin or extracts for seven days. Each mouse was anaesthetised with diethyl ether, and blood was drawn via retro-orbital puncture after 24 hours of treatment.

**Assessment of Biochemical Marker**

For further sample analysis, blood samples were then centrifuged for around 15 minutes at 3500rpm. The resulting serum was then analyzed using an analyzer to measure liver function marker enzymes, including ALT, ALP, and AST [28].

**Histopathological Studies**

Following blood collection, mice were euthanized. The liver was excised and weighed. The liver tissues were allowed to fix in 10% formalin. Samples from the right lobe were processed by sequential dehydration in graded ethanol concentrations, followed by clearing in xylene. The tissues were then embedded in paraffin wax using a tissue processor. The sections were cut using a microtome, stained with reagents, namely, eosin and hematoxylin, and examined for histological studies. To ensure objective histological assessment, two investigators who were blind to the therapy groups independently coded & assessed each liver segment. Using a semi-quantitative scoring method, the degree of liver injury was evaluated based on the degree of necrosis, inflammatory cell infiltration & regenerative alterations.

Liver histological preparations are observed under a light microscope with a magnification of 400 times. Calculation of the weight of liver histopathology change scores in different groups. Assessment of liver damage based on scoring criteria "Histology Activity Index (HAI - Knodell Score)."[29]

**Histology Activity Index (HAI - Knodell Score)**

**1. Scores for hepatocyte degeneration and necrosis**

| No. | The rate of change in the liver damage | Score |
|---|---|---|
| 1 | Normal | 1 |
| 2 | Damage is less than 1/3 of the liver lobules | 2 |
| 3 | Damage in 1/3 - 2/3 lobules of the liver | 3 |
| 4 | Damage > 2/3 of liver lobules | 4 |

When liver slices were examined histologically, the toxin control group showed significant structural changes, including cytoplasmic vacuolation, sinusoidal dilatation, inflammatory cell infiltration, and widespread hepatocellular necrosis. Normal sinusoidal gaps, intact hepatocyte cords, and prominent central veins were all features of the well-preserved hepatic architecture in the normal control group. On the other hand, hepatic architecture was restored in a dose-dependent manner following treatment with the plant extract.

While the low-dose group displayed a near-normal lobular structure with few necrotic foci and noticeable regenerative alterations, such as binucleated hepatocytes and enhanced mitotic figures, the extract group (400 mg/kg) demonstrated a moderate reduction in necrosis and minor regenerative activity. The majority of the liver is composed of hepatocytes.

Hepatocytes play a pivotal role in the liver's metabolism. These cells are situated in the space between the bile and the blood-filled sinusoids. The liver cell will undergo several morphological changes if it is injured by a variety of reasons that take place within the liver cell. These alterations may be deadly in the form of necrosis or sublethal, that is, degenerative.

**Statistical Analysis**

The results were expressed in mean ± SEM. The data were analysed using one-way ANOVA with Newman-Keuls multiple comparison test using GraphPad Instat 3 software.

## *RESULTS AND DISCUSSION*

### Result

**Botanical Evaluation**

The leaves were dark green and glossy. The adaxial surface appeared dark green, while the abaxial surface was pale green, turning reddish-green upon maturity. They were simple, elliptical in shape, with an entire margin, an acute apex, a narrow base, and reticulate venation, as shown in Figure 1.

The average leaf length ranged from 16 to 20 cm. Fresh leaves had a slightly bitter taste and no distinct odour. The transverse section of the leaf through the midrib reveals the presence of the vascular bundle, upper and lower epidermal cells, and a small pith located between the vascular bundle and the upper epidermis. The upper epidermis consists of a layer of parenchymatous cells. The lower epidermal cells are similar in size to those of the upper epidermis and are arranged in a single row. Beneath the upper epidermis, there are six to seven layers of parenchymatous cells. Within the midrib region, an oval-shaped cluster of parenchymatous cells is observed. The U-shaped collateral vascular bundle in the midrib consists of phloem on the outer side and xylem on the inner side, as illustrated in Figure 2.

**Physiological Evaluation**

Table 1 depicts the findings from the physicochemical study of *C. variegatum* leaf powder. The results indicated a moisture content of 12.3%, total ash of 4.05%, acid-insoluble ash of 0.4%, water-soluble ash of 0.7%, and 0.08% foreign matter. Furthermore, extractive values obtained using various solvents were as follows: ethanol (0.56%), petroleum ether (0.64%), chloroform (1.04%), benzene (14.72%) & aqueous extract (10.98%).

**Figure 1: Leaf of *Codiaeum variegatum***

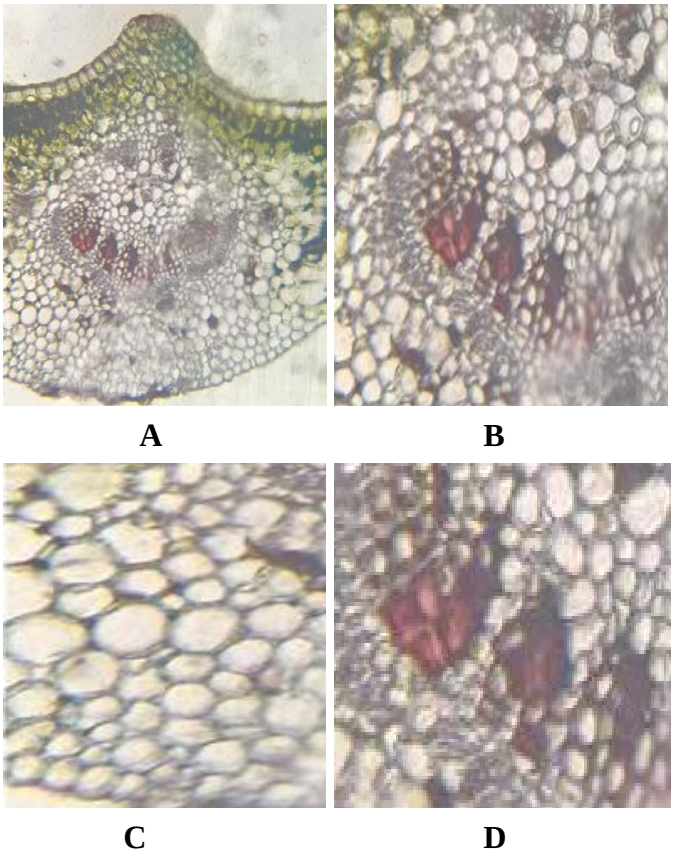



**Figure 2: Transverse section of *Codiaeum variegatum* leaf at 100x. A) Epidermis with paranchymatous cells; B) Vascular bundle arrangement; C) Collenchymatous cells; D) Collateral type vascular bundle, phloem (outer), xylem (inner).**

**Phytochemical Evaluation**

The chemical evaluation revealed the presence of various chemical constituents, including glycosides, alkaloids, saponins, fats, and oils, as listed in Table 2.

**Table 2: Physicochemical Evaluation**

| S.N | Physicochemical Parameter | Results |
|---|---|---|
| 1 | Loss on drying(%w/w) | 12.3 |
| 2 | Total ash value(%w/w) | 4.05 ± 0.14 |
| 3 | Acid insoluble ash value(%w/w) | 0.4 ± 0.05 |
| 4 | Water insoluble ash value(%w/w) | 0.7 ± 0.06 |
| 5 | Foreign organic matter | 0.08 |
| 6 | Extractive values(% yield | |
| a | Petroleum ether extract | 0.56 |
| b | Benzene extract | 0.64 |
| c | Chloroform extract | 1.04 |
| d | Ethanolic extract | 14.72 |
| e | Aqueous extract | 10.98 |

**Total Phenolic and Flavonoid Content**

Table 4 displays the total phenolic and flavonoid content of the ethanolic extract of *C. variegatum* leaves. The polyphenolic content of CV leaves is quantitatively estimated to be high in flavonoid and phenolic content. The procedure was performed using gallic acid and quercetin as standards [Figures 3 and 4]. The total phenolic content & total flavonoid content of croton leaves were 156 mg GAE/100 g and 334.15 mg QC/100 g, respectively.

**Table 3: Estimation of Polyphenolic Content**

| S.N | Ethanolic Extract | Results |
|---|---|---|
| 1 | Total Phenolic Content | 156 ±0.122 |
| 2 | Total Flavonoid Content | 334.15±0.16 |

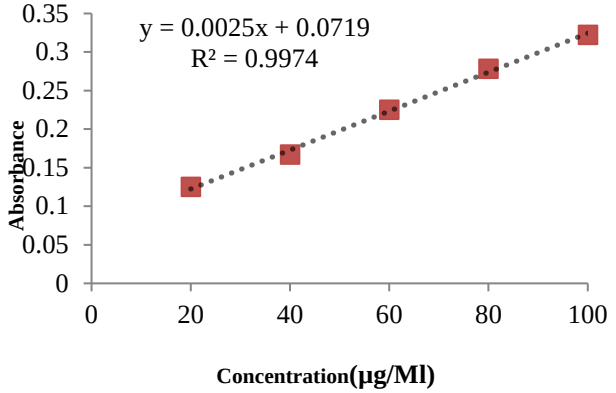



**Figure 3: Standard curve of Gallic acid**

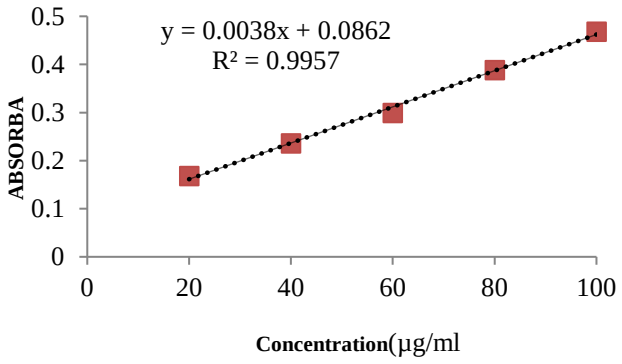



**Figure 4: Standard curve of Quercetin**

**ANTIOXIDANT ACTIVITY**

**DPPH Scavenging Assay**

The standard antioxidant, L-ascorbic acid, exhibited a free radical scavenging activity with an $IC_{50}$ value of 31.78 µg/mL, as shown in Figure 5. The plant extract demonstrated the ability to reduce DPPH-generated free radicals, with an $IC_{50}$ value of 115.22 µg/mL shown in Figure 6.

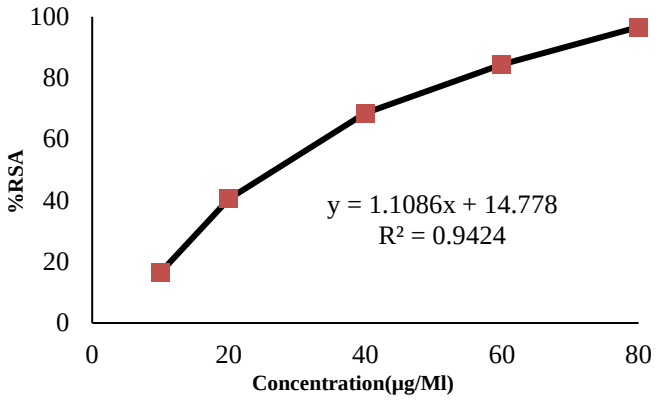



**Figure 5: DPPH scavenging assay for standard Ascorbic acid (% RSA Vs Concentration)**

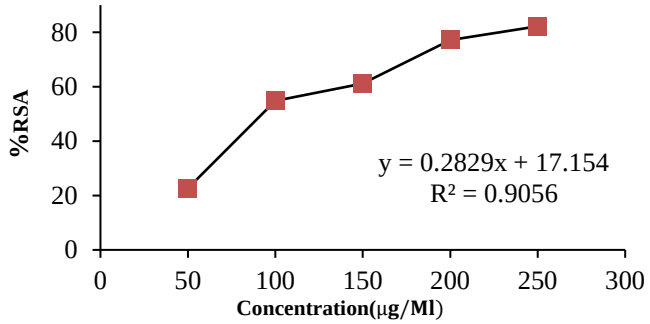



**Figure 6: DPPH scavenging assay of *Codiaeum variegatum* (% RSA Vs Concentration)**

**No Scavenging Assay**

The ascorbic acid exhibited a free radical scavenging activity of 27.32 µg/ml, shown in Figure 7, while the $IC_{50}$ of the ethanolic extract was determined to be 99.28 µg/ml shown in Figure 8.

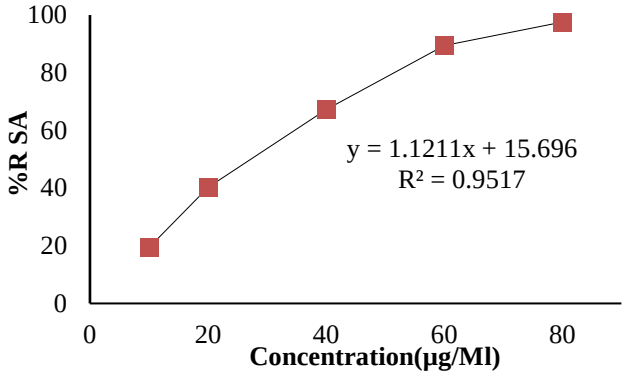



**Figure 7: NO scavenging assay for Standard Ascorbic acid (% Inhibition Vs Concentration)**

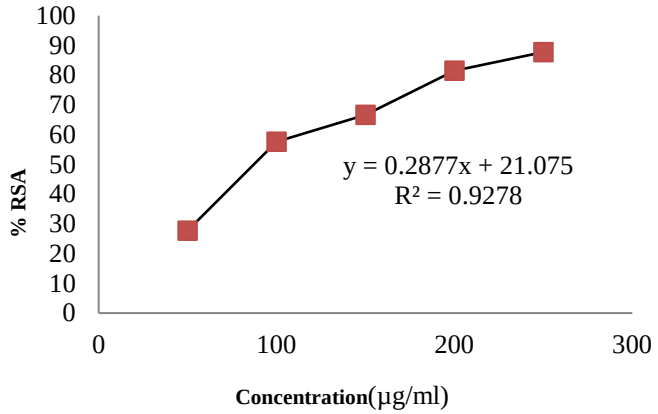



**Figure 8: NO scavenging assay of *Codiaeum variegatum* (% Inhibition Vs Concentration)**

**Hydrogen peroxide scavenging assay**

The standard antioxidant ascorbic acid showed an IC50 value of 32.22 µg/mL, as shown in Figure 9. The Inhibitory concentration value of the plant extract was estimated as 101.22µg/ml, as shown in Figure 10.

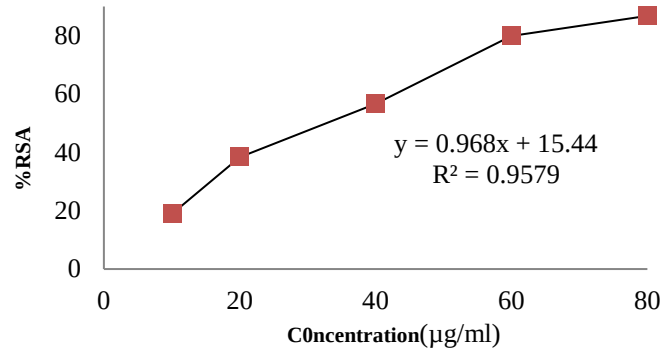



**Figure 9: $H_2O_2$ scavenging assay of standard Ascorbic acid (% Inhibition Vs Concentration)**

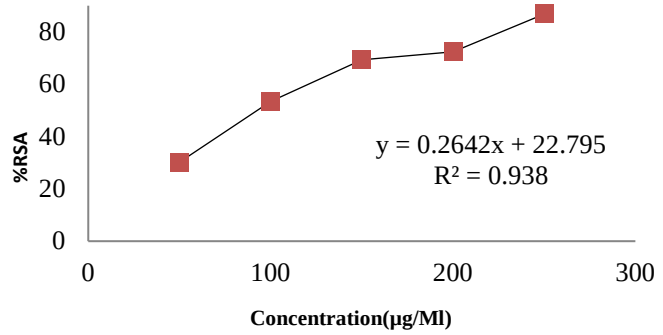



**Figure 10: $H_2O_2$ scavenging assay of *Codiaeum variegatum* (% Inhibition Vs Concentration)**

**Hepatoprotective Activity**

**Hepatotoxicity Index**

Compared to the control group, mice given only paracetamol (the toxic group) exhibited remarkable swelling in the liver and an increase in liver weight, indicating a marked rise in the hepatotoxicity index. In contrast, pre-treatment with 400 mg/kg of extract potentially reduced liver weight in the test group ($p < 0.05$). These findings highlight the hepatoprotective potential of the extracts shown in Figures 11 and 12, as well as Tables 5 and 6.

**Effect of Crude Extract on Liver Biomarker**

ALT, ALP, and AST levels were significantly higher in mice treated with paracetamol compared to the control group. Depending on the extract concentration, mice in those groups showed lower levels of these enzymes. Since enzyme values may return to normal during recovery, a shift toward control values suggests a healing effect. Mice treated with paracetamol had markedly increased levels of biomarkers, such as AST and ALT, compared to the control group ($p < 0.05$). In contrast, pre-treatment with two different doses of the ethanolic extract of Codiaeum variegatum significantly ($p < 0.05$) reduced both ALT and AST levels compared to the disease control and standard. Additionally, the ethanolic extract significantly ($p < 0.05$) lowered ALP levels at all tested doses compared to the disease control and standard groups, as shown in Figures 12, 13, and 14, and Table 6.

**Effect of Crude Extract on Liver Cell Degeneration and Necrosis**

Ethanol crude extract can reduce the score of degeneration and necrosis of liver cells induced by paracetamol. Degeneration scores in the normal control, Disease control EECV1, EECV2, and standard groups were 21.6%, 37.4%, 17.8%, 19.5%, and 26.2%, respectively. The percentage of degeneration and necrosis is shown in Table 7.

**Histological Analysis**

The parenchymal architecture of the liver tissues in the control group (A) appeared intact, with no signs of cellular necrosis. In contrast, mice treated with paracetamol alone (B) displayed congested blood vessels, infiltration of inflammatory cells, and necrotic as well as hyperaemic hepatocytes. The silymarin-treated group (C) showed restored hepatocyte arrangement and repaired sinusoidal cells. Mice receiving 400 mg/kg of the crude extract (D) exhibited normal hepatocytes and clear signs of liver cell regeneration, which was more pronounced than in the group treated with 200 mg/kg of extract (E). Mitotic markers confirmed renewed liver cell growth in the treated groups, shown in Figure 15

**Table 4: Phytochemical Evaluation**

| Name of Identification test | Ethanolic Extract |
|---|---|
| **1. Alkaloids** | |
| a)Wagner's test | + |
| b)Hager's test | + |
| c)Myers's test | + |
| **2. Glycosides** | |
| a)Keller Kiliani test | - |
| b) Borntrager test | - |
| c) Legal test | + |
| **3. Flavonoids** | |
| a)Shinoda test | ++ |
| b) Lead acetate test | + |
| **4. Saponin** | |
| a)Foam test | - |
| b)Forth test | - |

| Name of Identification test | Ethanolic Extract |
|---|---|
| **5. Carbohydrates** | |
| a)Molish test | - |
| b) Benedict test | - |
| **6. Tannin** | |
| a) Ferric Chloride test | + |
| **7. Fat and Oil** | |
| a) Saponification test | - |
| b) Filter Paper test | - |
| **8. Protein** | |
| a) Ninhydrine test | + |
| b) Biuret test | + |

*Chemical identification of Codiaeum varigetum extract "++": positive test and "-"negative test*

**Table 5: Estimation of Hepatotoxicity index in diff. group**

| Weight | Normal Control | Diseases control | PCM+Standard (Silymarin, 100mg/kg) | $EECV_1$ (200mg/kg,P.O) | $EECV_2$ (400mg/kg, P.O) |
|---|---|---|---|---|---|
| **Weight of mice** | 25.75± 0.75 | 26.70 ± 0.57 | $29.78 \pm 0.46^{b**}$ | $31.77 \pm 0.47^{b***c*}$ | $29.3\pm0.32^{b**}$ |
| **Weight of liver** | 323.6± 3.47 | $900.8\pm16.10^{a***}$ | $539.6 \pm2.71^{b***}$ | $777.7\pm9.28^{b***c***}$ | $613.0\pm3.86^{b***c***d***}$ |
| **Hepatotoxicity index** | 20.51± 0.75 | $41.39\pm1.28^{a***}$ | $22.54\pm 0.58^{b***}$ | $33.11\pm1.07^{b***c***}$ | $27.61\pm0.7^{b***c***d***}$ |

*Values are expressed as mean ± SD (n = 6). Data analyzed using One way-ANOVA with Newman-keuls Multiple comparison test ***P<0.001, ** P<0.05,* P<0.01; [a] When compared to Normal control; [b] When compared to PCM (Disease control); [c] When compared to PCM + silymarin (100mg/kg); [d] When compared to PCM+ EECV, p.o). PCM: Paracetamol; EECV: ethanolic extract of Codiaeum varigetum. Leaves*

**Table 6: Effect of AST, ALP, and ASP on diff. treated group**

| Biochemical Marker | Normal Control | Diseases control | PCM+Standard (Silymarin, 100mg/kg) | $EECV_1$ (200mg/kg,P.O) | $EECV_2$ (400mg/kg, P.O) |
|---|---|---|---|---|---|
| **AST(IU/L)** | 74.82 ± 3.11 | $379.1 \pm 6.53^{a***}$ | $95.92 \pm 1.03^{b***}$ | $204.6\pm4.98^{b***c***}$ | $174.9 \pm 5.41^{b***c***d***}$ |
| **ALT(IU/L)** | 33.29 ± 0.78 | $180.2 \pm 3.31^{a***}$ | $74.10\pm0.73^{b***}$ | $144.2 \pm 1.29^{b***c***}$ | $129.3 \pm 2.21^{b***c***d***}$ |
| **ALP(IU/L)** | 64.89± 0.70 | $176.5 \pm 3.02^{a***}$ | $75.49 \pm 0.96^{b***}$ | $144.0 \pm 2.07^{b***c***}$ | $126.1 \pm 1.25^{b***c***d***}$ |

*Values are expressed as mean ± SD (n = 6). Data analyzed using one-way ANOVA with Newman-Keuls multiple comparison test ***P<0.001, ** P<0.05,* P<0.01; [a] When compared to Normal control; [b] When compared to PCM (Disease control); [c] When compared to PCM + silymarin (100mg/kg); [d] When compared to PCM + EECV, (p.o). PCM: Paracetamol; EECV: ethanolic extract of Codiaeum varigetum. Leaves*

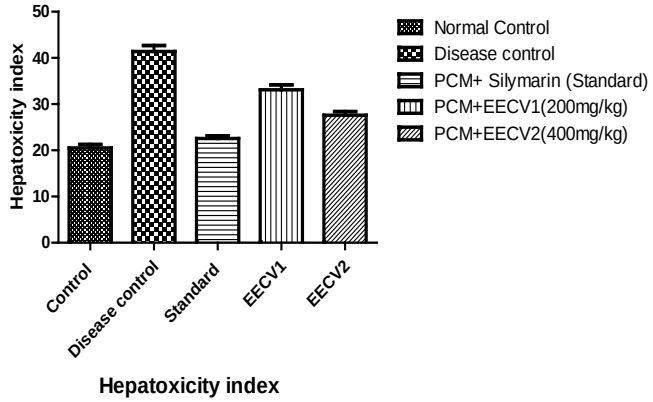



**Figure 11: Effect of hepatotoxicity on different treated group**

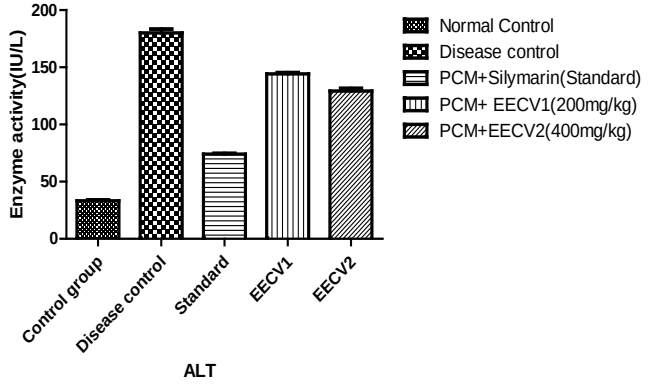



**Figure 12: Effect of ALT biochemical parameter on different treated groups**

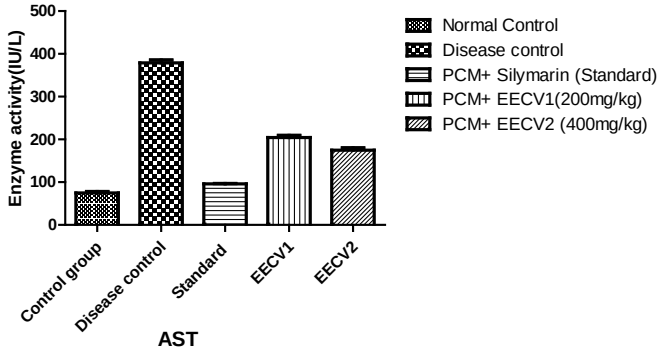



**Figure 13: Effect of AST biochemical parameter on different treated groups**

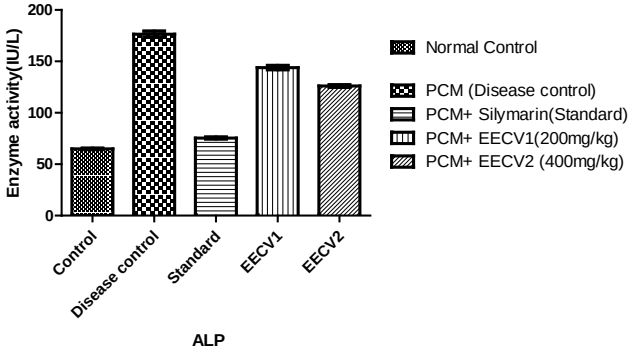
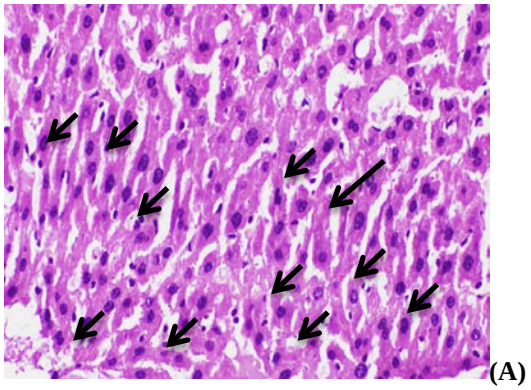


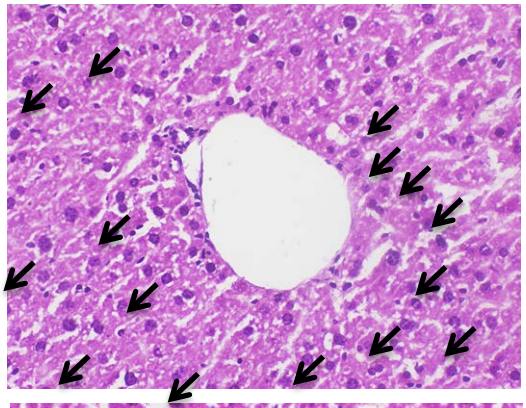

**Figure 14: Effect of ALP biochemical parameter on different treated groups**

**Table 7: Estimation of degeneration and necrosis score in different groups**

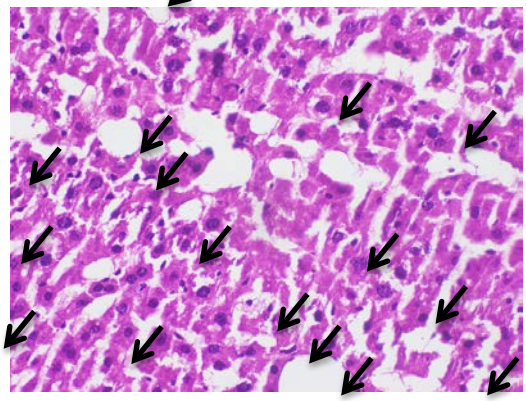
| | Normal control | Disease control | $EECV_1$ | EECV2 | PCM+ Silymarin |
|---|---|---|---|---|---|
| **Degeneration/Necrosis** | 21.6% | 37.4% | 17.8% | 19.5% | 26.2% |
| **Score** | 1 | 2 | 1 | 1 | 1 |



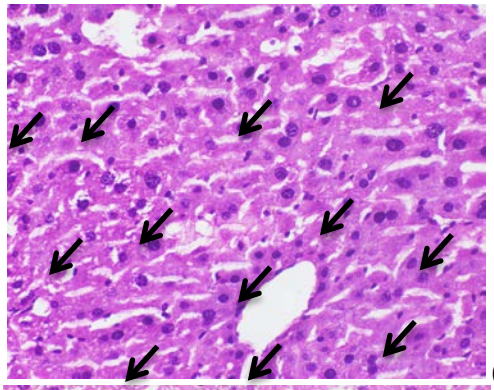
**(A)**



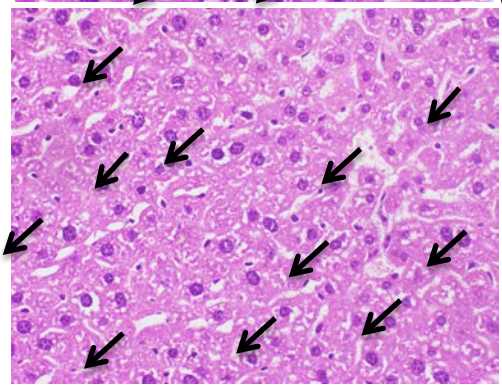
**(B)**



**(C)**



**(D)**



**(E)**

**Figure 15: Histological sections with Hematoxylin and eosin dye under 400X. Arrow indicating the degenerating/necrotic cells (A) Control group: normal cellular architecture with distinct hepatic cells, sinusoids lining cells, blood vessels; (B) PCM treated group shows apoptotic, Necrosed hepatocytes and blood vessels with near inflammation; (C) PCM+ Silymarin treated group: Hepatocytes with uniform nucleus and cytoplasm; (D) PCM+ EECV (200mg/kg) and (E) PCM+ EECV (400mg/kg): preserved hepatic architecture, minimized necrosis with occasional vacuolation in the cells.; EECV: Ethanolic Leaf extract of *Codiaeum variegatum*: PCM: Paracetamol.**

***DISCUSSION***

Paracetamol-induced hepatotoxicity is a widely accepted model for evaluating hepatoprotective agents. Liver damage typically leads to elevated levels of key biochemical parameters due to the leakage of these enzymes into the bloodstream following hepatic injury [30]. Additionally, liver dysfunction impairs protein synthesis by disrupting polyribosomes on the endoplasmic reticulum, leading to reduced levels of total protein and albumin. When crude plant extracts are used, variations in phytochemical content resulting from harvest season, plant age, and extraction conditions can cause batch-to-batch variability. The absence of pure compound testing compromises mechanistic clarity and restricts our capacity to link reported bioactivities to particular bioactive elements.

The lack of in vitro–in vivo correlation and dose-response optimization limits the data's predictive potential and translational significance. We aim to avoid overinterpreting our findings and provide a fair perspective by presenting these issues in detail. Future research directions, such as chemical separation, standardization of extracts, and mechanistic validation, are also highlighted by these limitations. Specifically, compared to many common ornamental plants, *Codiaeum variegatum* has a somewhat higher phenolic and flavonoid content (156 mg GAE/100 g and 334.15 mg QE/100 g, respectively). The TPC and TFC of Mirabilis jalapa flower extract were 175.02 mg GAE/100 g and 616.40 mg QE/100 g, respectively [31]. This implies that bioactive compounds with antioxidant qualities may be abundant in ornamental plants.

The antioxidant activity of the extract was confirmed through DPPH, NO, and $H_2O_2$ scavenging assays, with $IC_{50}$ values of 115.22, 99.28, and 101.22 µg/mL, respectively. The extract demonstrated a dose-dependent activity. This antioxidant activity is likely due to the presence of active metabolites such as polyphenols and flavonoids. Despite the extract's remarkable ability to scavenge and reduce free radicals, its antioxidant efficiency was consistently inferior to that of ascorbic acid, the reference component. Numerous causes contribute to this discrepancy. As a pure, low-molecular-weight substance with a distinct and very effective redox potential, ascorbic acid may directly neutralize reactive oxygen species (ROS). In contrast, the plant extract comprises a complex variety of bioactive elements, among which only a few may contribute significantly to antioxidant activity.

Liver toxicity was also associated with an increase in liver weight, considered a hepatotoxicity index. The mice administered paracetamol showed an increase in liver weight, which may be attributed to intracellular water accumulation and hepatocyte swelling. However, administration of 400 mg/kg of *Codiaeum variegatum* ethanol extract significantly decreased the liver weight in mice, indicating hepatoprotective potential. Biochemical analysis revealed that pretreatment with the extract at different doses significantly reduced the elevated levels of ALT, ALP, and AST induced by paracetamol.

These findings reveal that the extract helps preserve the functional and structural integrity of the liver by stabilizing hepatocyte membranes and possibly restoring normal biliary function, as evidenced by a reduction in ALP levels. The most significant level of hepatic necrosis and degeneration (37.4%) was observed in the disease control group, indicating that the hepatotoxin had caused liver damage. With equivalent histological scores of 1, treatment with the ethanol extract of Codiaeum variegatum (EECV1 and EECV2) significantly decreased the degree of liver damage to 17.8% and 19.5%, respectively, suggesting significant hepatoprotection. The protection was similar to that of the group typically treated with silymarin. These results support the hepatoprotective potential of *C. variegatum* extract, indicating that it efficiently reduces liver cell necrosis and degeneration.

One proposed mechanism behind the hepatoprotective action involves inhibition of the cytochrome P450 enzyme CYP2E1, which is responsible for converting paracetamol into its toxic metabolite NAPQI. Overproduction of NAPQI depletes hepatic glutathione, leading to oxidative stress, lipid peroxidation, and cellular damage. For the antioxidant mechanism, the Nrf2 pathway is responsible. Usually, Nrf2 remains in the cytoplasm after forming a complex with the actin-bound inhibitor protein Keap1. In the presence of oxidative stress, the Nrf2-Keap1 interaction is disrupted in a dose-dependent manner, allowing Nrf2 to enter the nucleus & combine with the small musculoaponeurotic fibrosarcoma protein to form a heterodimer. Therefore, this complex activates the ARE pathway, which regulates the trans- cription of multiple antioxidant & detoxification genes that eliminate toxins & carcinogens before they have a chance to damage cells [32]. The crude extract may enhance glutathione levels, thereby mitigating oxidative damage and preserving liver function.

The hepatoprotective effects observed can be attributed to various bioactive constituents present in the extract, including polyphenols, flavonoids, alkaloids, saponins, and coumarins. Previous studies have identified several compounds from *C. variegatum*, such as neoclerodan-5,10-en-19,6β; trachyloban-19-oic acid, and 20,12-diolide, 3α,19-dihydroxytrachylobane, which may contribute to its biological activity [33]. Histopathological evaluations further supported these findings. Mice treated with paracetamol displayed hepatic necrosis, inflammatory infiltration, and vascular congestion, typical features of liver damage. In contrast, tissues from mice treated with the plant extract or silymarin showed potential histological improvement, indicating protection against paracetamol-induced hepatocellular injury.

## *CONCLUSION*

The ethnomedical importance of Codiaeum variegatum (croton leaves) is justified by the proven ethanol extract being highly hepatoprotective and antioxidant in nature, as presented in the present manuscript. The high levels of total phenolic content (TPC) and total flavonoid content (TFC) present in the extract indicate an important capacity to scavenge free radicals. This underscores the essential role that polyphenols and flavonoids play in mitigating liver damage caused by oxidative stress. The additional indications suggest that these bioactive compounds are the primary mediators of the hepatoprotective effect, including the positive correlation between phytochemical richness and antioxidant activities. Crucially, the study indicates that Croton leaves may serve as a natural source of hepatoprotective compounds, supporting the long-standing assertion that they can be used to treat liver-related conditions. However, crude extracts are not standardized by nature, even though they provide initial therapeutic insight. To verify their effectiveness and safety, future research should concentrate on the structural characterization, separation, and purification of individual phytoconstituents in addition to dose-response and pharmacokinetic analyses. Assessment of oxidative stress biomarkers, hepatic enzyme regulation, and molecular signaling pathways are examples of mechanistic evaluations required to determine how these chemicals function as protective agents. All the results indicate the potential of *C. variegatum* leaves as a source of new hepatoprotective compounds. However, before their therapeutic use can be confirmed and converted into evidence-based natural therapies, thorough phytochemical and mechanistic research is necessary.

## *FINANCIAL ASSISTANCE*

NIL

## *CONFLICT OF INTEREST*

The authors declare no conflict of interest.

## *AUTHOR CONTRIBUTION*

Swapnil Pandey contributed to the development of the technique, execution of the experimental data, writing of the original draft, and data analysis. Sonia Singh supervised the research and provided critical insights. She also contributed to the manuscript review.

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