Pharmacological investigations of *Graptophyllum pictum* as hepatoprotective potential in albino rats

Mamta Goswami and Harikesh Maurya*

Siddhartha Institution of Pharmacy, I.T. Park, Sahastradhara Road, Dehradun, India

ABSTRACT

Objective: The present study was designed to evaluate preliminary phytochemical and pharmacological evaluations of the whole plant of *Graptophyllum pictum* aqueous extract for hepatoprotective and *in-vitro* antioxidant activities. **Method:** Wistar rats were divided into five groups having six animals in each. Group I served as normal control and orally administered normal saline; Group II (disease control) rats were orally administered with Paracetamol (2 gm/kg b.w.); Group III (standard control) rats were administered with Silymarin (100 mg/kg p.o.); Group IV and V rats were orally administered with aqueous extract of *Graptophyllum pictum* at low dose (200 mg/kg b.w.) and high dose (400mg/kg b.w.) respectively once a day for two weeks. **Results:** The qualitative chemical tests revealed the presence of carbohydrates, tannins, phenolic compounds, alkaloids, glycosides, flavonoids, and steroids along with few inorganic constituents i.e. iron, sulphate, phosphate and chloride. The obtained results for such an activity of *G. pictum* (400 mg/kg) were SGPT (83.32±0.93 IU/L), SGOT (163.91±0.91 IU/L), direct bilirubin (0.73±0.008 mg/dl), total bilirubin (0.82±0.006 mg/dl) and triglycerides (177.16±0.63 mg/dl) shows significant reductions as compared to the disease control. The histology of liver tissue reported that the high dose (400 mg/kg) showing the normal and well defined cytoplasm, nucleus and central hepatic vein. The antioxidant activity like *in-vitro* DPPH assay, favonoid content, total phenolic content, ABTS radical scavenging activity, hydrogen peroxide scavenging method confirms the significant hepatoprotective potency. **Conclusion:** Thus, the investigations revealed that whole plant of *Graptophyllum pictum* possessed potent hepatoprotective and antioxidant activities.

Keywords: *Graptophyllum pictum,* Hepatoprotective, Antioxidant, DPPH assay, ABTS radical scavenging activity.

Introduction

Medicinal plants have key position in traditional medicine system utilized for health and therapeutic benefits across the world [1,2]. It has been estimated that approximately 50% of the modern drugs have been originated from natural products [3]. Recently, WHO has been reported that before the development of modern medicine, herbal drugs being contained in therapeutic practices that have been in continuation often for hundreds of years are still in use today [4,5].
The *Graptophyllum pictum belonging to* family Acanthaceae (caricature) is a tropical evergreen shrub that grows to 6-9 inch tall [6].

**Correspondence*

Harikesh Maurya
Associate Professor
Siddhartha Institute of Pharmacy,
Near I.T. Park, Sahastradhara Road,
Dehradun-248001, (UK) India
E-Mail: mauryaharikesh2@gmail.com

The leaves are oval to elliptic, deep green varyingly blotched with cream along the mid veins. The flowers are 3-4 inch long, red to purple-red colour, tubular with protruding stamens and bloom in summer [7].
Graptophyllum pictum has been found to contain alkaloids, glycosides, pectin, formic acid, steroids, saponins, tannins, flavonoids & alcohol [8]. This plant is used in treatment of reducing fertility, constipation, rheumatism, hemorrhoid, urinary infections and scabies, maturing boil process, smoothing skin, hepatomegaly and ear diseases [9]. It also has anti-fungal, anti-inflammatory, anti-plaque, laxative, anti-viral, anti-bacterial properties [10].
Liver failure is the impairment of all functions of liver that affect the related activities of the body [11]. It may be acute or chronic depend on varieties of disorders, such as; acute viral hepatitis, severe necrosis due to poisonings, cirrhosis, medical procedures including abdominal paracentesis and portacaval shunt operations [12]. Jaundice is yellowish discoloration of the skin and mucous membrane due to excessive formation of bilirubin than the normal liver [13,14].

Bilirubin, a catabolic product of heame, is conjugated in the liver to form bilirubin diglucuronide and excreted in bile. The normal concentration of serum bilirubin ranges from 0.2- 1mg/dl. Overproduction of bilirubin (2-2.25 mg/dl), stored in the blood, diffuses into the tissues which then become yellow. This condition is called jaundice or icterus [15,16]. Most of the conventional liver function tests do not reflect the capacity of the liver metabolizes and a poor correlation exists between the extent of hepatic dysfunction and attenuated drug metabolism. A number of drugs like CCl_4, paracetamol, halothane, methyldopa, isoniazid, allopurinol are known to induce hepatotoxicity [17].

It was reported that about 20,000 deaths occur every year due to liver diseases globally. In India, the main causes of hepatic diseases are excessive intake of drugs, polluted atmosphere and alcohol consumption. Over 40 polyherbal pharmaceutical preparations reputed to have hepatoprotective action are being used. Medicinal plants have drawn much attention for the treatment of liver disorders as hepatoprotective agents [18].

Materials and Methods

Collection and Authentication of plant: The plant material was collected from the Krishnendra nursery near Lalbagh, Bangalore (India) in the month of March 2015. The plant was authenticated as *Graptophyllum pictum* (Acanthaceae), the provided reference no. NISCAIR/RHMD/Consult/2015/2876/69 by botanical scientist at NISCAIR, Raw Material Herbarium and Museum Delhi (RHMD), India.

Phytochemical evaluation: The plant material was dried under controlled conditions to avoid appreciable chemical changes and mechanically reduced to coarse powder for further extraction. The whole plant was subjected to defatting followed by extraction with water as solvent [19].

Extraction through cold maceration: Coarsely powdered drug was subjected to cold maceration using water as a solvent. The extract was filtered, concentrated to a small volume in rotary vacuum evaporator followed by drying in china dishes on hot water bath. The extract was then weighed and percentage yield was calculated and the obtained extracts were further used for phytochemical and pharmacological investigation [20].

Preliminary phytochemical screening: The phytochemical study was performed through the powder drug subjected for various organic solvents of increasing polarity. Various extracts *viz* petroleum ether, chloroform, ethyl acetate, methanolic and aqueous solvent were subjected to qualitative chemical analysis in order to confirm the presence or absence of various phytoconstituents such as; alkaloids, carbohydrates, glycosides, tannins, phenolic compounds and flavonoids [21].

Procurement of animals: With prior approval from the institutional Animal Committee, the albino rats (either sex) were procured from the institute animal house and maintained at 24-28°C. They were kept in large polypropylene cages and well-ventilated animal house with 12/12 hrs dark and light cycle. The animals were acclimatized for two week under laboratory condition and maintained as per CPCSEA guideline.

Procurement drugs and kit: Diagnostic kits used for estimation of SGPT, SGOT, Bilirubin and triglycerides were obtained from Span Diagnostics Ltd. The chemical (LR grade) used in the study was purchase from local supplier. Paracetamol (PCM) was used as inducing hepatotoxicity in rats.

Biomarkers: The following serum parameters were estimated for the hepatoprotective activity was serum glutamate oxaloacetate transaminase (ASAT), serum glutamate pyruvate transaminase (ALAT), serum triglycerides, and Serum bilirubin. Blood was withdrawn by retro orbital puncture for the determination of biochemical parameters. Rats were dissected at the end of study and liver was collected, washed, and used for histopathological studies [22].

Experimental design: All animals, housed in standard hygienic conditions cages, were fed with a standard diet and water. Wistar rats were further divided into five groups having six animals in each. Group I served as normal control and orally administered normal saline; Group II (disease control) rats were orally administered with Paracetamol (2 gm/kg b.w.); Group III (standard control) rats were administered with Silymarin (100mg/kg p.o.); Group IV and V rats were orally administered with aqueous extract of *G. pictum* at low dose (200mg/kg b.w.) and high dose (400mg/kg b.w.) respectively once a day for two weeks.

Antioxidant activity

DPPH radical scavenging activity assay: The free radical scavenging activity of the aqueous extract of *Graptophyllum pictum* was monitored *in-vitro* by 2,2'-diphenyl-1-picrylhydrazyl assay. The stock solution, prepared by dissolving DPPH (20 mg) with methanol (100 ml) was stored at 20 ^{0}C. The working reagent was

made by diluting DPPH solution with methanol to attain an absorbance of about 0.98±0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (100-500µg/ml). The mixture was shaken, incubated for 15 min and absorbance was taken at 517 nm. The control was prepared as similar without any sample [23]. The scavenging activity was calculated by using following formula:

$$\% \text{ inhibition} = \frac{\text{Ac } 517 - \text{At } 517}{\text{Ac } 517 \text{nm}} \times 100$$

Where, Ac = Absorbance of control; At =Absorbance of test

Hydrogen peroxide scavenging activity: Hydrogen peroxide solution (2 mm) was prepared in 50 mm phosphate buffer (pH 7.4). The volume of test tubes containing 0.1ml of different fractions of aqueous extract was adjusted up to 0.4 ml with 50 mM phosphate buffer (pH 7.4). After addition of 0.6 ml H_2O_2 solution, tubes were vortexed and absorbance was determined at 230 nm after 10 minutes, against a blank solution containing phosphate buffer [24]. Hydrogen peroxide scavenging activity was calculated by given formula:

$$\% \text{ inhibition} = \frac{1 - \text{At } 230}{\text{Ac } 230 \text{nm}} \times 100$$

Where, Ac = Absorbance of control; At =Absorbance of test

ABTS radical scavenging activity: The 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) solution (7 mM) was treated with potassium persulfate (2.45 mM) solution and kept for overnight in a dark to yield a dark coloured solution containg ABTS radical cation. The dark coloured solution was diluted with 50% methanol for an initial absorbance of about 0.70±0.02 at 745 nm. Free radical scavenging activity was assessed by mixing 300µl of test sample with 3 ml of ABTS working standard in a microcuvette. The decrease in absorbance was measured exactly one minute after mixing the solution, then up to 6 min [25]. the percentage inhibition was calculated according to the formula.

$$\% \text{ inhibition} = \frac{\text{Ac } 517 - \text{At } 517}{\text{Ac } 517 \text{nm}} \times 100$$

Where, Ac = Absorbance of control; At =Absorbance of test

Estimation of total phenolic content: 1 ml of sample (aqueous extract of *G. pictum* (1mg/ml) was mixed with 1 ml of Folin ciocalteu's phenol reagent. After 5 min, 10 ml of 7% Na_2CO_3 solution and 13 ml of deionized distilled water was added and mixed thoroughly. The mixture was kept in the dark for 90 min at 23 °C, after which the absorbance was read at 750 nm [26]. The Total phenolic content was determined from extrapolation of calibration curve made by preparing gallic acid solution.

Estimation of total flavonoid content: 0.3 ml of aqueous extract of *G. pictum* 3.4 ml of 30% methanol, 0.15 ml of $NaNO_2$ (0.5M) and 0.15 ml of $AlCl_3.6H_2O$ (0.3M) were mixed in a test tube. After 5 min, 1 ml of NaOH (1M) was added. The solution was mixed well and the absorbance was measured against the reagent blank at 506 nm. The standard curve for total flavonoids was made using rutin standard solution (0-100 mg/l) under the same procedure [26].

Statistical analysis: Statistical analysis was carried out using Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA). The results were expressed as mean±SEM. Statistical significance between more than two groups was tested using one-way ANOVA followed by Turkey's multiple comparison tests. Values of $p<0.05$ were considered significant.

Results and Discussion

Extraction and phytochemical investigation: Extraction was prepared in water and percentage yield obtained as 19.4%. The phytochemical screening for organic constituents revealed that the extracts indicated the presence of tannins, alkaloids, glycosides and flavonoids except steroids. Whereas, the inorganic elements *viz* sulphate, phosphate, chloride were also found in the extract. However, the drug was devoid of magnesium, iron and carbonates. The other inorganic constituents such as magnesium, iron, sulphate, phosphate, chloride, and carbonate were also identified.

Hepatoprotective activity: Hepatoprotective activity of aqueous extract of *G. pictum* was investigated against paracetamol induced hepatotoxicity in albino rats. The results of hepatoprotective activity are given in table 1.

Table 1: Effect of *G. pictum* aqueous extract on different biochemical parameters in rats

Treatments	SGPT (IU/L)	SGOT (IU/L)	Direct Bilirubin (mg/dl)	Total Bilirubin (mg/dl)	Triglyceride (mg/dl)
Normal saline (1ml/kg)	65.07±0.33	134.5±0.58	0.30±0.034	0.59±0.020	90.92±2.56
PCM (2 g/Kg b.w.)	116.34±0.37#	235.28±0.73#	1.44±0.190#	1.19±0.039#	177.16±0.63#
Silymarin 100 mg/kg + PCM	74.00±2.55*	149.39±2.09*	0.52±0.071*	0.78±0.028	122.79±1.58*
Low dose of extract 200 mg/kg + PCM	95.59±0.51	186.25±0.91	0.61±0.005*	0.92±0.006	145.20±1.28
Low dose of extract 400 mg/kg + PCM	83.32±0.93*	163.91±0.91*	0.73±0.008*	0.82±0.006	125.91±4.49*

Values are expressed as mean±SEM (n=6); #p<0.05 as compared to normal control, *p<0.05 as compared to PCM control.

The histopathological studies provided supportive evidence for biochemical parameters. Histology of liver section of normal control rats (group I) showed the normal histological architecture with well defined cytoplasm, nucleus and central vein (Fig. 1), while the positive control group showed severe damage, hepatic necrosis and fatty degenerations of hepatocytes. Treatment with aqueous extract recovered the injured liver to quite normal.

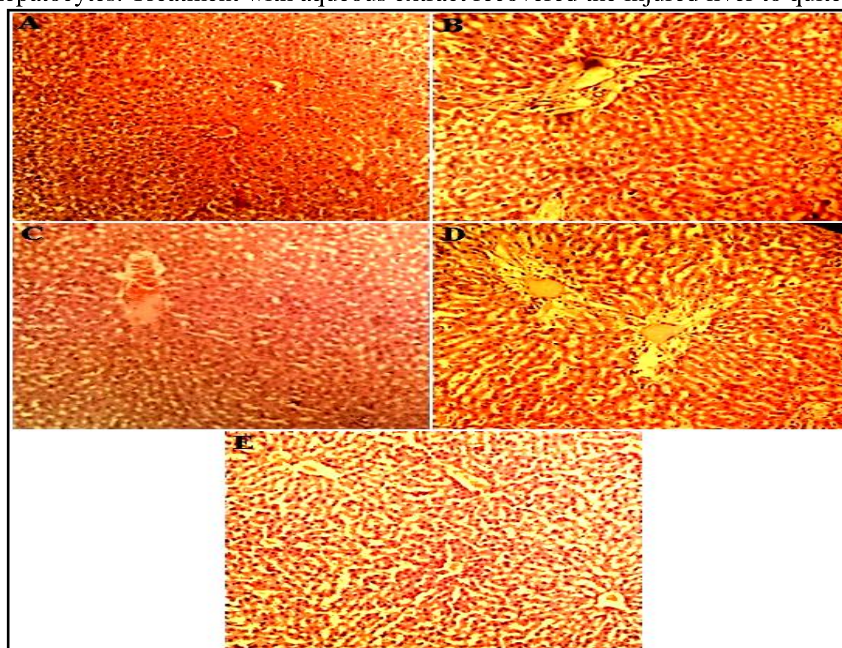

Figure 1: Histopathology of liver tissue of entire groups of rat stained by hematoxylin and eosin. (A) Liver section of normal control rats showing central vein that chords of hepatocytes radiating without damage; (B) PCM group showing severe damage in central vein, and changes of fatty degenerations as well as necrosis of hepatocytes; (C) Silymarin treated group showing central vein with mild to moderate diffuse granular degeneration and very mild necrosis in hepatocytes; (D) Liver of rat treated with extract at 200 mg/kg showing regeneration of hepatocytes and reduced necrosis; (E) Liver of rat treated with extract at 400 mg/kg showing light regeneration of hepatocytes and reduced necrosis.

Paracetamol is a common antipyretic agent that is safe in therapeutic doses, but can produce fatal hepatic necrosis in man, rats and mice with toxic doses. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome or depletion of hepatic glutathione is a prerequisite for PCM-induced hepatotoxicity. Due to liver damage, cellular leakage and loss of functional integrity results elevated serum enzymes levels.

The experimental findings of hepatoprotective activity of aqueous extract of *Graptophyllum pictum* exhibited remarkable protective effect against the paracetamol induced hepatotoxicity in rats. Administration of paracetamol (2 g/kg, b.w) to each group except control group caused elevation of serum enzyme levels (SGOT, SGPT), direct bilirubin, total bilirubin and triglycerides as compared to control group.

The aqueous extracts (AEGP) at low dose (200 mg/kg b.w) and high dose (400 mg/kg b.w) showed significant reduction in elevated levels of serum enzymes (SGOT, SGPT), direct bilirubin, total bilirubin and triglycerides. The effects were comparable to that of standard drug silymarin (100 mg/kg b.w). The aqueous extract at dose level (400 mg/kg b.w) was found to be more potent as compared to low dose (200 mg/kg b.w).

IN–VITRO Antioxidant Activity

Antioxidant potential of aqueous extract of *Graptophyllum pictum* (whole plant) was examined using different methods i.e., DPPH radical scavenging, hydrogen peroxide (H_2O_2) scavenging, total phenolic content, ABTS and total flavanoid content.

DPPH radical scavenging activity: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of aqueous extract of *G. pictum* was determined by using ascorbic acid as standard. The inhibition data were recorded against the selected concentration 100-500 (µg/ml). The % inhibition curves for ascorbic acid (Fig. 2) and that for *G. pictum* aqueous extract (Fig. 3) were plotted from which IC_{50} value of DPPH by ascorbic acid and *G. pictum* aqueous extract was calculated by using regression equation mentioned in table 2.

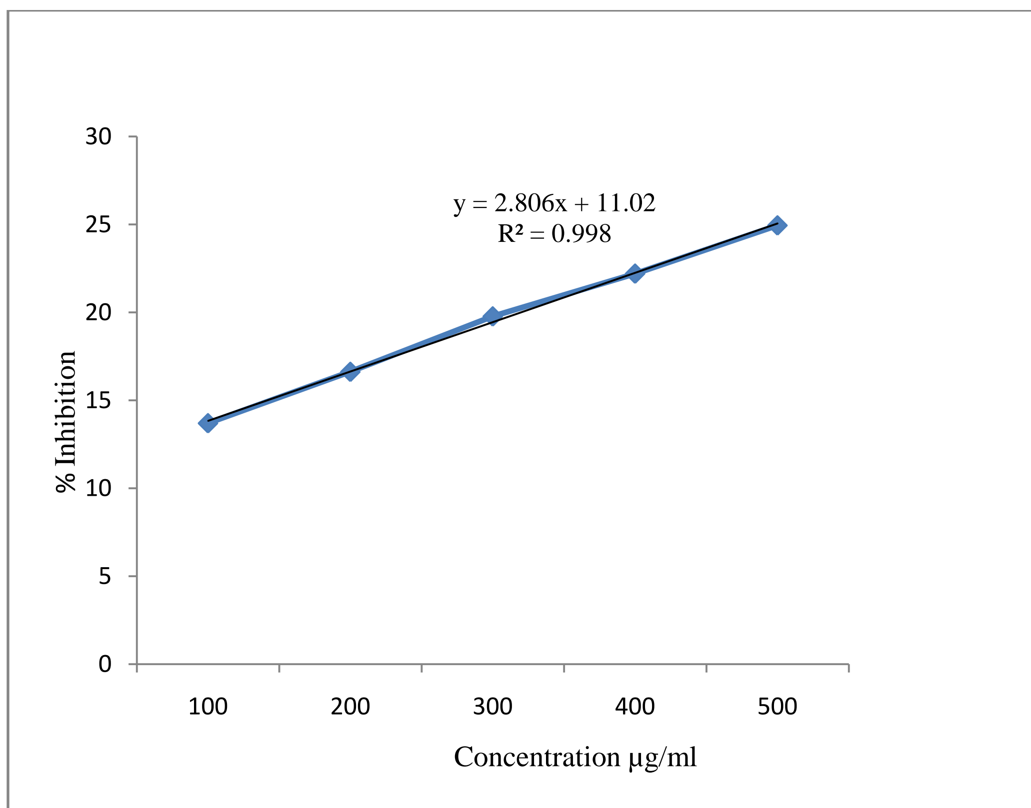

Figure 2: Standard curve for DPPH assay of ascorbic acid

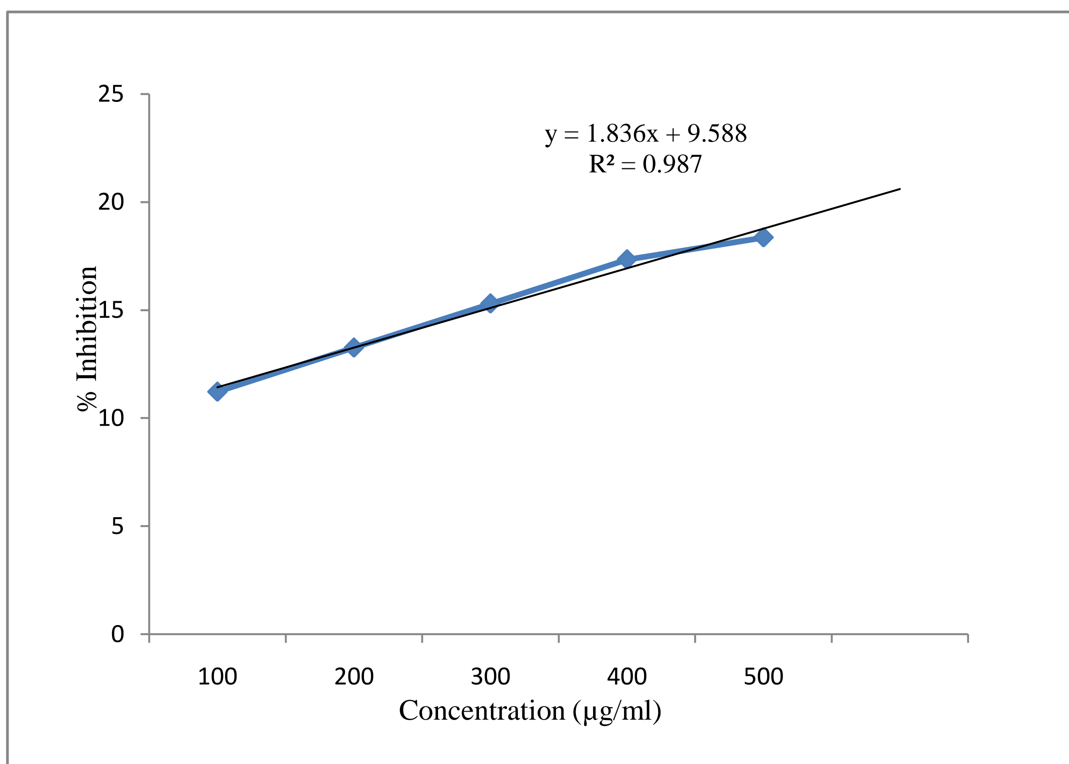

Figure 3: Standard curve for DPPH assay of aqueous extract of G. pictum

Table 2: IC_{50} data of ascorbic acid and aqueous extract of *G. pictum*

S. No	Sample	IC_{50}
1.	Ascorbic acid	13.89 µg/ml
2.	Aqueous extract *of G. pictum*	22.01 µg/ml

The study showed that IC_{50} values of DPPH assay for ascorbic acid and *G. pictum* aqueous extract were 13.89µg/ml and 22.0µg/ml respectively. The study observed that the aqueous extract was active in DPPH radical scavenging. At concentration extract 100-500 µg/ml, R^2 value was found to be 0.987 which could be considered as best fit one. Line of regression was found to be 1.836x + 9.588 which revealed IC_{50}=22.01µg/ml. The effect of antioxidant on DPPH radical scavenging was thought to be due to their hydrogen donating ability.

Estimation of total flavonoids content: Total flavonoids content of *Graptophyllum pictum* aqueous extract was estimated by using rutin solution as standard. The absorbance data were recorded against the selected concentration 50-300 µg/ml. The standard curve was plotted from which flavonoids contents of the *G. pictum* aqueous extract (Fig. 4) the values was calculated using regression equation.

H_2O_2 radical scavenging activity: Hydrogen peroxide (H_2O_2) radical scavenging activity of *Graptophyllum pictum* aqueous extract was estimated by using ascorbic acid solution as standard. The inhibition data was recorded against the selected concentration 100-500 µg/ml. The standard inhibition curve for hydrogen peroxide radical scavenging of ascorbic acid (Fig. 5) and inhibition curve for *G. pictum* aqueous extract (Fig. 6) were plotted. From these IC_{50} values of H_2O_2 radical scavenging of ascorbic acid and *G. pictum* aqueous extract were calculated using regression equation mentioned in table 3.

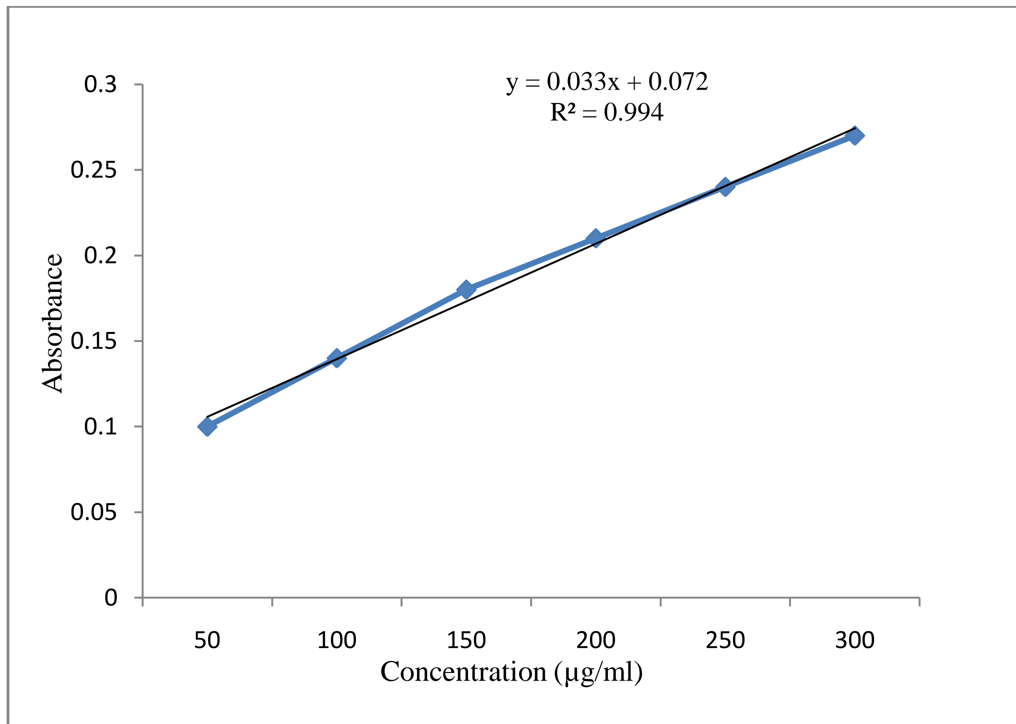

Figure 4: Standard curve for total flavonoids content using rutin as standard

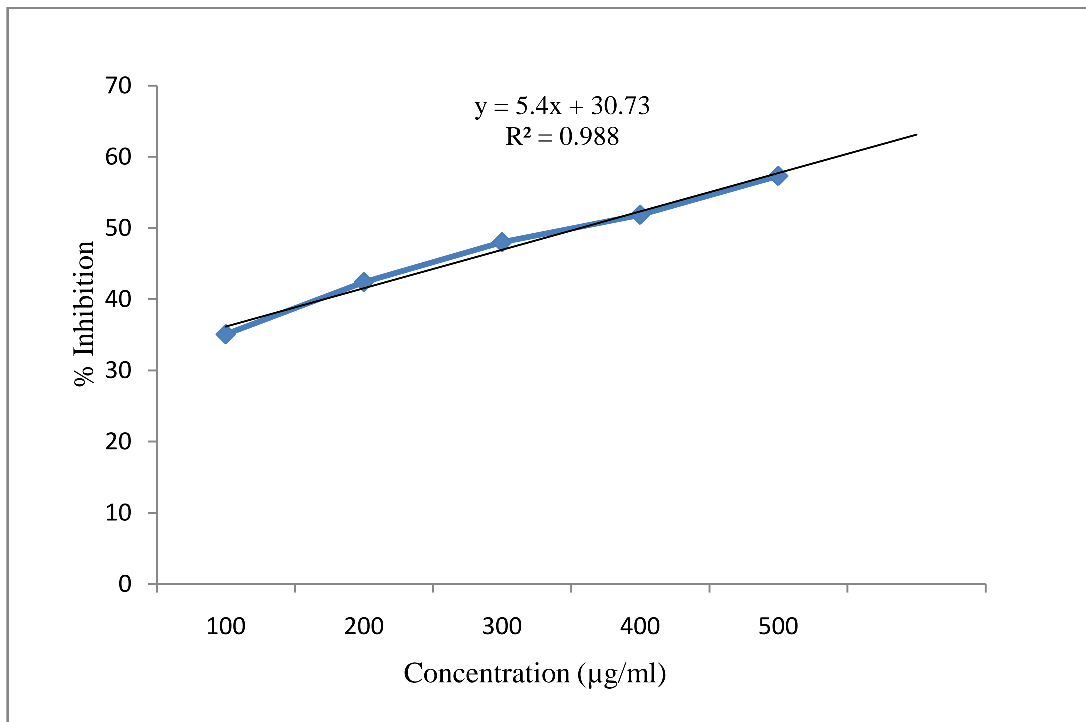

Figure 5: Standard curve for hydrogen peroxide scavenging activity by ascorbic acid

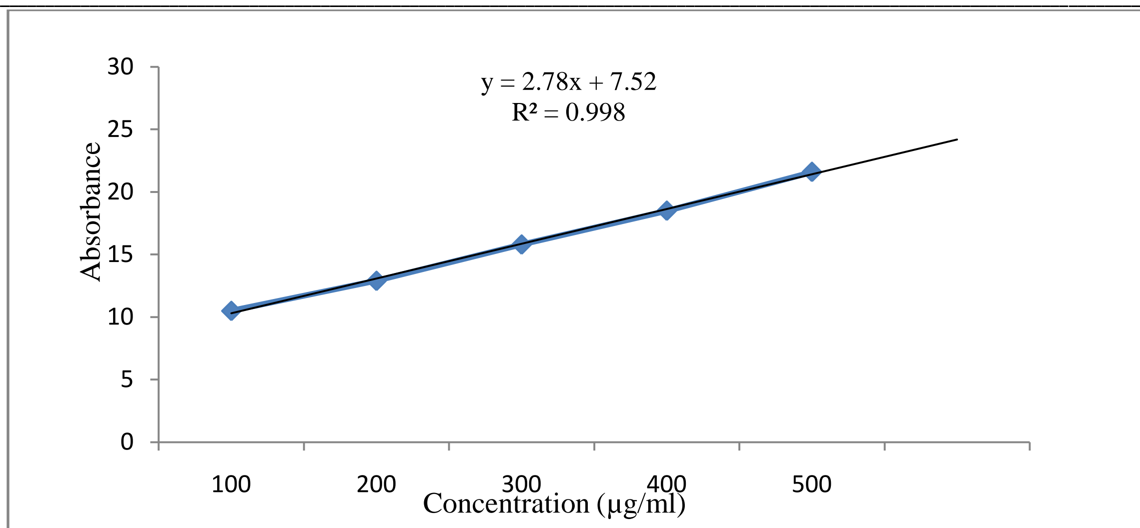

Figure 6: Standard curve for H_2O_2 scavenging activity of aqueous extract of G. pictum

Table 3: IC_{50} values of H_2O_2 and aqueous extract of *G. pictum*

S. No	Sample	IC_{50}
1.	Ascorbic acid	3.56 µg/ml
2.	Aqueous extract *of G. pictum*	15.28 µg/ml

The study showed that IC_{50} values of H_2O_2 radical scavenging activity for ascorbic acid and *G. pictum* aqueous extract were 3.56 µg/ml and 15.28 µg/ml respectively. The study observed that ascorbic acid revealed greater activity of H_2O_2radical scavenging as compared to aqueous extract of whole plant of *G. pictum*. At concentration 100-500 µg/ml for *G. pictum* of aqueous extract, R^2 value was found to be 0.998 which could be considered as best fit one. Line of regression was found to be 2.78x + 7.52 which revealed IC_{50} = 15.28µg/ml.

Total phenolic content: Total phenolic content of *G. pictum* aqueous extract was estimated by using gallic acid solution as standard. The absorbance data were recorded against the selected concentration 50-250 µg/ml. The standard curve was plotted from which phenolic content of *G. pictum* aqueous extract (Fig. 7) was calculated using regression equation.

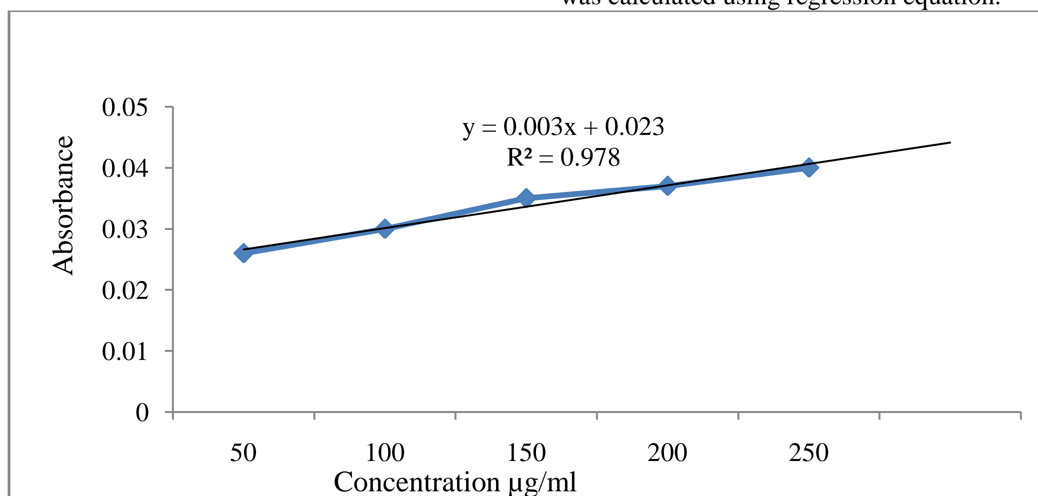

Figure 7: Standard curve for total phenolic content of gallic acid

ABTS radical scavenging activity: ABTS (2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)) scavenging activity of *G. pictum* aqueous extract was estimated by using ascorbic acid as standard. The inhibition data were recorded against the selected concentration 50-300 µg/ml. The % inhibition curves for ABTS (Fig. 8) and *G. pictum* extract (Fig. 9) were plotted from which IC_{50} value of ABTS and *G. pictum* aqueous extract was calculated using regression equation (Table 4).

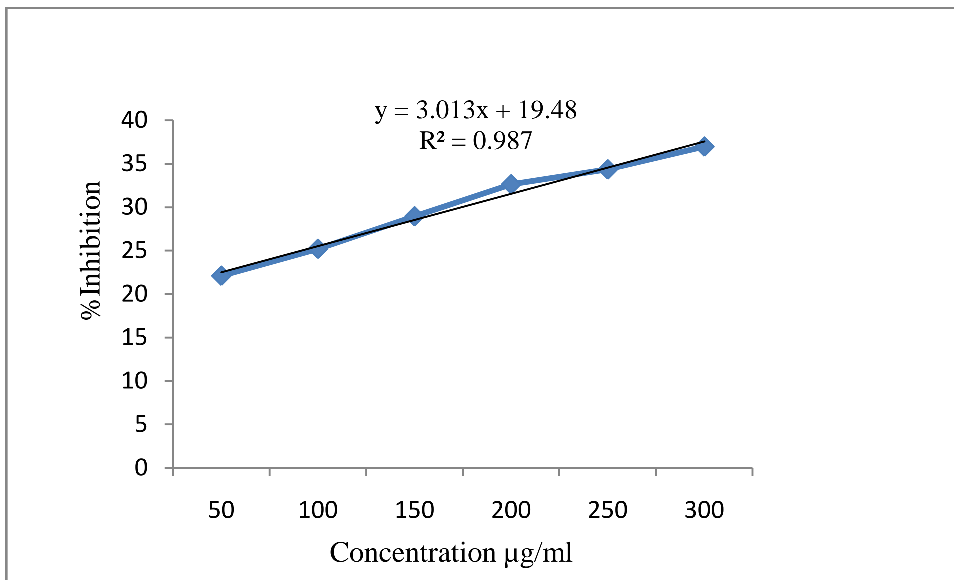

Figure 8: Standards curve for ABTS radical scavenging activity

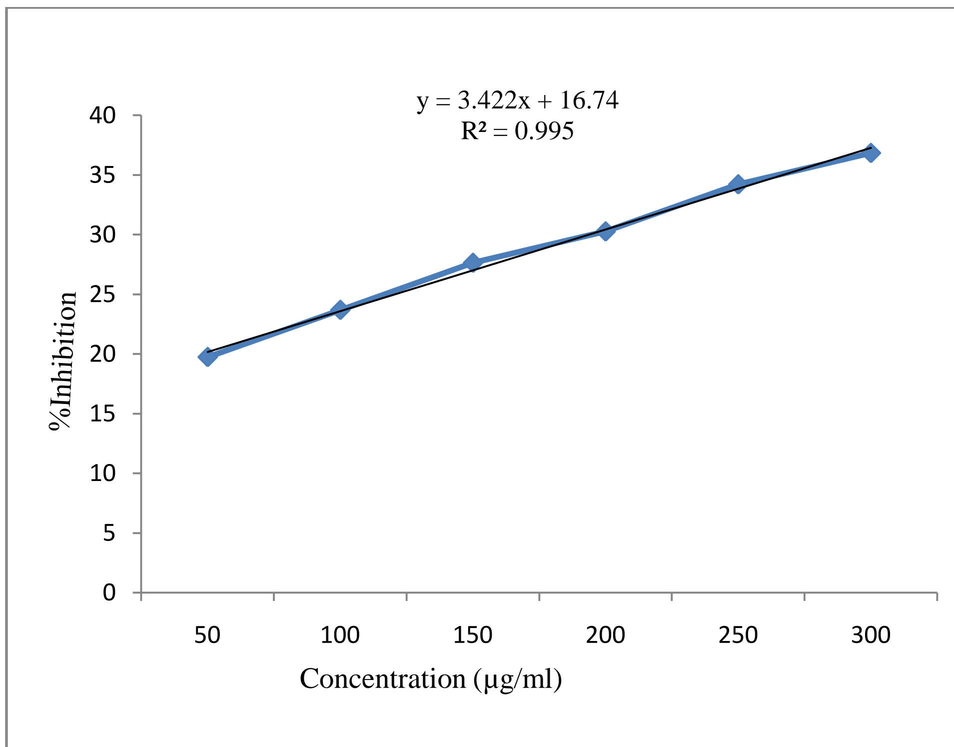

Figure 9: Standards curve for ABTS radical scavenging activity of G. pictum

Table 4: IC_{50} data of ABTS aqueous extract of *G. pictum*

S. No	Sample	IC_{50}
1.	Ascorbic acid	10.13µg/ml
2.	Aqueous extract *G. pictum*	19.78µg/ml

The study showed that IC_{50} values of ABTS assay and *G. pictum* aqueous extract were 10.13µg/ml and 19.78 µg/ml respectively. In this present study, it was observed that ascorbic acid revealed greater activity of ABTS radical scavenging as compared to aqueous extract of *G. pictum.* At concentration 50-300 µg/ml, R^2 value was found to be 0.995. Line of regression was found to be 3.422x + 16.74 which revealed IC_{50} = 19.78µg/ml.

The fresh aqueous extract of *Graptophyllum pictum* (whole plant) was subjected for pharmacological studies *viz* hepatoprotective and *in-vitro* antioxidant activities. *G. pictum* aqueous extract observed as significant hepatoprotective potential when subjected to paracetamol induced hepatotoxicity test. The obtained results for such activity of *G. pictum* (400 mg/kg) were as follows; SGPT (83.32±0.93), SGOT (163.91±0.91), direct bilirubin (0.73±0.008), total bilirubin (0.82±0.006) and triglycerides (125.91±4.49) showing significant reductions as compared to the disease control group of rat.

The aqueous extract *of G. pictum* established antioxidant activity by using *in-vitro* DPPH assay, flavanoid content, total phenolic content, ABTS radical scavenging activity, and hydrogen peroxide (H_2O_2) scavenging method. The % inhibition for aqueous extract in DPPH assay method was estimated 11.22, 13.26, 15.30, 17.34 and 18.36 at various concentrations i.e., 100, 200, 300, 400, and 500 µg/ml respectively. The result for total flavonoids content of aqueous extract of *G. pictum* was found to be 1.54µg/ml. The % inhibition of aqueous extract at various concentrations (100, 200, 300, 400, and 500 µg/ml) were 10.05, 12.9, 15.8, 18.5 and 21.6 respectively. The obtained result for total phenolic content of aqueous extract *G. pictum* was found to be 3.33µg/ml. In ABTS method, the % inhibition of aqueous extract at various concentrations (50, 100, 150, 200, 250 and 300µg/ml) was 19.73, 27.63, 30.26, 34.21 and 36.84 respectively.

CONCLUSION

The whole plant extract of *Graptophyllum pictum* revealed significant hepatoprotective activity on paracetamol induced hepatotoxicity in rat model. *G. pictum* extract confirmed significant antioxidant activity when subjected to in-vitro DPPH assay, flavonoids content, H_2O_2 scavenging activity, total phenolic content and ABTS scavenging method. These investigations revealed that *G. pictum* possess a potent hepatoprotective and antioxidant activities. However, further research on detailed pharmacological screening, isolation of active phytoconstituents responsible for therapeutic activity and clinical study for evaluation of safety and efficacy of the drug, need to be assessed.

Acknowledgments

The authors are thankful to the Chairman, Director and Principal of the Siddhartha Institute of Pharmacy, Near I.T. Park, Sahastradhara Road, Dehradun (India) for providing necessary facilities to carry out the research work peacefully.

Declaration of interest

All the authors have no conflict of interest.

REFERENCES

1. Veeresham C. Natural products derived from plants as a source of drugs. J Adv Pharm Technol Res 2012;3(4):200–01.
2. Pandey MM, Rastogi S, Rawat AKS. Indian traditional Ayurvedic system of medicine and nutritional supplementation. Evid Based Compl Altern Med 2013;2013:1–12.
3. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis. Bull World Health Organ 2008;86(8):594–99.
4. World Health Organization. World Health Organization, Geneva: WHO Traditional Medicine Strategy, 2013; p. 2014–23.
5. Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. Molecules 2016;21(559):1-18.
6. *Graptophyllum,* Caricature Plant. Nursery live, Access from-https://nurserylive.com/buy-annual-flowers-plants-online-in-india/graptophyllum-caricature-plant-plants-in-india.
7. *Graptophyllum pictum*. Missouri Botanical Garden, access from-http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a519.

8. Wahyuningtyas E. The Graptophyllum pictum extract effect on acrylic resin complete denture plaque growth. Maj Ked Gigi 2005;38(4):201–04.
9. Keng SB, Lim M. Denture plaque distribution and the effectiveness of a perborate-containing denture cleanser. Quintessence Int 1996;27(5):341-45.
10. Singh P, Khosa RL, Mishra G, Tahseen MA. A phytopharmacological review on Justicia picta (Acanthaceae): A well known tropical folklore medicinal plant. J Coastal Life Med 2015;3(12):1000-02.
11. Giulia-Anna P. Ascites in patients with cirrhosis. Can Fam Physician 2013;59(12):1297-99.
12. Van-Wagner LB, Green RM. Evaluating Elevated Bilirubin Levels in Asymptomatic Adults. JAMA 2015;313(5):516–17.
13. Stillman AE. Chapter 87-Jaundice. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd edn, Walker HK, Hall WD, Hurst JW, editors. Boston: Butterworths; 1990.
14. Lamont JT, Isselbacher KJ. Postoperative jaundice. N Engl J Med 1973;288:305–57.
15. Erlinger S, Arias IM, Dhumeaux D. Inherited disorders of bilirubin transport and conjugation: New insights into molecular mechanisms and consequences. Gastroenterology 2014;146(7):1625-38.
16. Chowdhury JR, Chowdhury NR, Wu G, Shouval R, Arias IM. Bilirubin mono- and diglucuronide formation by human liver in vitro: assay by high-pressure liquid chromatography. Hepatology 1981;1(6):622-27.
17. Barstow L, Small RE. Liver function assessment by drug metabolism. Pharmacotherapy 1990;10(4):280-88.
18. Vuittonet CL, Halse M, Leggio L, Fricchione SB, Brickley M, *et al.* Pharmacotherapy for alcoholic patients with alcoholic liver disease. Am J Health Syst Pharm 2014;71(15):1265–76.
19. Devi P, Meera R, Muthumani P, Kameswari B, Badmanaban R. Phyto-Physico chemical evaluation and Antioxidant activities of leaves of *Naphellium lappaceum*. J Pharm Sci Res 2009;1(3):117-22.
20. Kumar D, Bhat ZA, Kumar V, Chashoo IA, Khan NA, *et al*. Pharmacognostical and phytochemical evaluation of Angelica archangelica Linn. Int J Drug Dev Res 2011;3(3):173-88.
21. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *in vitro* antioxidant activities. Asian Pac J Trop Biomed 2014;4(1):S359–67.
22. Kamei T, Asano K, Nakamura S. Determination of serum glutamate oxaloacetate transaminase and glutamate pyruvate transaminase by using L-glutamate oxidase. Chem Pharm Bull (Tokyo) 1986;34(1):409-12.
23. Ilahi I, Samar S, Khan I, Ahmad I. *In vitro* antioxidant activities of four medicinal plants on the basis of DPPH free radical scavenging. Pak J Pharm Sci 2013;26(5):949-52.
24. Ashoka Babu VL, Arunachalam G, Jayaveera KN, Madhavan V, Banu S. Free radical scavenging activity of methanolic extract of *Ecbolium viride* (Forssk). Alston roots. Der Pharmacia Lettre 2011:3(4):285-88.
25. Collins P, Dobson ADW, Field JA. Reduction of the 2,2′-Azinobis(3-Ethylbenzthiazoline-6-Sulfonate) Cation Radical by Physiological Organic Acids in the Absence and Presence of Manganese. Appl Environ Microbiol 1998;64(6):2026–31.
26. Gokbulut I, Bilenler T, Karabulut I. Determination of Chemical Composition, Total Phenolic, Antimicrobial, and Antioxidant Activities of Echinophora tenuifolia Essential Oil. Int J Food Proper 2013;16:1442-51.

Source of Support: Nil
Conflict of Interest: None