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In-vitro hepatoprotective activity of Pagoda (Clerodendrum paniculatum) leaves

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ABSTRACT: Clerodendrum paniculatum is reported to have ethnomedicinal importance as the plant is used as remedy for ailments and disorders such as wounds, typhoid, snakebite, jaundice, giddiness, malaria, anemia and hemorrhoids. Objective: This research point to assess the antioxidant and hepatoprotective activity of Pagoda (Clerodendrum paniculatum) leaves. Materials and Methods: Extracts of Clerodendrum paniculatum leaves were subjected to antioxidant activity (DPPH, Hydroxyl scavenging and Nitric oxide scavenging), cytotoxicity study by MTT assay and in vitro hepatoprotective activity using HepG2 cell line culture. Results: The ethanolic extract exhibited a good potential to act as a free radical scavenger for DPPH inhibition, hydroxyl scavenging and nitric oxide scavenging activity. **T**he ethanolic extract of Clerodendrum paniculatum leaves also showed a better in vitro hepatoprotective activity in Paracetamol induced hepatotoxixity in HepG2 cell line culture.

KEYWORDS: Clerodendrum paniculatum, DPPH, Hydroxyl radical scavenging, MTT assay, Hepatoprotective.

I. INTRODUCTION

Medicinal plants stand a better chance of providing potent, safer, affordable, and easily accessible therapies for oxidative stress-related ailments. Medicinal plants contain various secondary metabolites, which have demonstrated a wide spectrum of pharmacologic activities. Antioxidant properties of plants have been demonstrated to play a protective role in the body against diseases, since their consumption lowers the risk of cancer, heart disease, hypertension, dementia, and stroke **(1).**

The liver performs the normal metabolic homeostasis of the body as well as biotransformation, detoxification and excretion of many endogenous and exogenous compounds, including pharmaceutical and environmental chemicals. Drug-induced hepatotoxicity is a major cause of iatrogenic diseases. The World Health Organization (WHO) has laid emphasis on promoting the use of traditional medicine for health care. Hence, identifying medicinal plants with ethano botanical significance and folklore eminence should be considered as an essential factor in order to encourage the use of herbal medicines and to reveal their potential as source of new drugs **(2)**.

Plants and natural products have been used traditionally worldwide for the prevention and treatment of liver disease. Scientific research has supported the claims of the medicinal efficacy of several of these herbal compounds, as evidenced from the voluminous work on their hepatoprotective potentials (**3)**. Clerodendrum paniculatum is one of the important species of the genus Clerodendrum L. distributed in many Asian countries. The plant was first described in 1767 by the 'father' of modern biological nomenclature – the Swedish botanist, Carl Linnaeus. The species epithet refers to the large 'paniculate' clusters of flowers (inflorescence), the feature which makes this such a visually-striking plant (**4)**. The ability of some species of Clerodendrum genus in counteracting free radicals using the DPPH method is very good in C. serratum, C.infortunatum, C. Inerme, C. Viscosum and C. phlomidis **(5).** Clerodendrum paniculatum is reported to have ethnomedicinal importance as the plant is used as remedy for ailments and disorders such as wounds, typhoid, snakebite, jaundice, giddiness, malaria, anemia and hemorrhoids. Various phytochemicals such as rutin, quercetin, β-sitosterol, β-amyrin, lupeol, oleanolic aldehyde acetate, stigmasta-4,25-dien-3-one, and (3β)-stigmasta-4,22,25-trien-3-ol have been identified in this plant. The plant is shown to exhibit biological activities such as antimicrobial, antioxidant, anthelmintic, anti-inflammatory, antimutagenic, cytotoxic, hypolipidemic, insecticidal and anti-ageing activity **(6)**. This article is about the details of antioxidant

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and in vitro hepatoprotective activity of Clerodendrum paniculatum leaves.

II. MATERIALS AND METHOD

Collection

Leaves of Clerodendrum paniculatum were collected from the surroundings of Karkala, Karnataka, India. The collected leaves were authenticated by Dr. K.P. Srinath, Professor in Botany, Bangalore University, Bengaluru, India (Voucher number - VB2).

Extraction

The leaves were dried in shade, ground into powder and successively extracted with solvents such as petroleum ether, chloroform, ethyl acetate, ethanol and water using soxhlet apparatus. The extracts were dried. The concentrated extracts were stored at 4°C until further use.

Preliminary phytochemical investigation

Qualitative tests for various phytochemicals present in the extracts of Clerodendrum paniculatum leaves were carried out using standard phytochemical screening procedures (**7-9)**. Visual examination was done for the appearance of colour, precipitation or frothing, which was used as an indicator for the presence or absence of a given phytochemical group.

In-vitro evaluation of antioxidant activity

DPPH scavenging activity

The stable 1, 1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for the determination of free radical-scavenging activity of the extract. Various concentrations (25µg, 50µg, 75µg and 100µg) of extracts were added to required volume of ethanol to make up to 1.5ml. Further 1.5ml of DPPH (0.1mM in ethanol) solution was added and the mixture was placed in the dark at room temperature for 30min. The absorbance was measured at 517 nm against the corresponding blank solution which is prepared by taking sample and ethanol and control was prepared by taking ethanol and 1.5ml DPPH solution. Ascorbic acid minute intervals by mixing 1.0mL incubation mixture with an equal amount of Griess reagent. The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 546 nm. All tests were performed in duplicates. Ascorbic acid was used as positive control. The % inhibition was calculated as follows with 25 µg concentration was used as standard **(10,11)**. The assay was performed in duplicates.

Percentage inhibition of free radical DPPH was calculated based on control reading by following equation

DPPH scavenged (%) = $(A_c - A_t)/ A_c \times 100$

A_c - is the absorbance of the control reaction, A_t - is the absorbance in the presence of the sample of the extracts.

IC_{50} value was calculated by using formula: $IC_{50}= (\sum C/\sum I) X 50$

Where, $\sum C$ is the sum of extract concentrations used to test and $\sum I$ is the sum of the % of inhibition at different concentrations**.**

Hydroxyl radical scavenging activity

The extracts were subjected to hydroxyl radical scavenging activity (**12)**. The reaction mixture 3.0 ml contained 1.0 ml of 1.5mM $FeSO_4$, 0.7 ml of 6mM hydrogen peroxide, 0.3 ml of 20mM sodium salicylate, and varying concentrations of all extracts. After incubation for 1 hour at 37ºC the absence of the hydroxylated salicylate complex was measured at 562 nm. Ascorbic acid was used as standard. The percentage scavenging effect was calculated as

Scavenging activity = [1-(A1-A2)/A0] × 100%

Where A0 was the absorbance of the control (containing all reagents except the sample extract), A1 was the absorbance in the presence of the extract, and A2 was the absorbance without sodium salicylate.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was carried out where the nitric oxide radical generated from sodium nitroprusside was measured (**13)**. The reaction mixture (5.0 mL) containing sodium nitroprusside (5 mM) in phosphate-buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25°C for 3 hours. The nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion, which was assayed at 30-

% Inhibition = (Ao-At) / Ao x 100

Where Ao is the absorbance of the control (blank, without extract) and At is the absorbance in the presence of the extract.

Total Phenolic content

The total phenolic content in ethanolic extract was determined by using Folin-Ciocalteu colourimetric method based on oxidation-reduction

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reaction (**14,15)**. Various concentrations of gallic acid solutions in methanol (10, 25, 50 and 75 µg/ml) were prepared. In five different 10 ml volumetric flasks, 1 ml gallic acid of each concentration was added and to that 5 mL of Folin-Ciocalteu reagent (10%) and 4mL of 7.5 % Na_2CO_3 were added to get a total volume of 10 ml. The blue coloured mixture was shaken well and incubated for 30 minutes at room temperature. Then the absorbance was measured at 765 nm against blank. All the experiments were carried out in duplicate. The average absorbance values obtained at different concentrations of gallic acid were used to plot the calibration curve. Total phenolic content of the extract was calculated from the regression equation of calibration curve and expressed as mg gallic acid equivalents (GE) per gram of sample in dry weight (mg/g).

Total flavonoid content

Estimation of total flavonoid content in ethanolic extract was done by Aluminum chloride colorimetric method (**16)**. 0.5ml of ethanolic extract (1mg/ml) in methanol was mixed with 1.5ml of methanol, 0.1ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 10 to 100 µg/ml in methanol. All the experiments were carried out in duplicate. Total flavonoid content of the extract was calculated from the regression equation of calibration curve and expressed as mg quercetin equivalent (QE) per gram of sample in dry weight (mg/g).

Cytotoxicity study (MTT assay)

Cytotoxicity study was done using HepG2 cell line by MTT assay (**17)**. 32mg/ml stocks of extracts were prepared using DMSO. Serial two fold dilutions were prepared from 20µg/ml to 0.312µg/ml using Dulbecco's Modified Eagle's Medium (DMEM) media for treatment. HepG2 cell line was procured from ATCC (American Type Culture Collection- Cell Biology Collection bioresource), stock cells was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells/well were seeded in a 96 well plate and incubated for 24hrs at 37°C, 5% CO_2 incubator. The monolayer cell culture was trypsinized and the cell count was adjusted to 5 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well Microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. After 24hrs, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different test concentrations of test drugs were added on to the partial monolayer in Microtiter plates. Doxorubicin was used as control. The plates were then incubated at 37°C for 24hrs in 5% CO_2 atmosphere. After incubation the test solutions in the wells were discarded and 100 µl of MTT (5mg/10 ml of MTT in PBS) was added to each well. The plates were again incubated for 4hrs at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line.

% Inhibition = ((OD of Control – OD of sample)/OD of Control) x 100

The morphological changes in the HepG2 cells for control (untreated) and test (treated with 10µg/ml and 320µg/ml concentration of extracts) were observed. The results are shown in (Fig1).

In-vitro hepatoprotective activity

Hepatoprotective activity was carried out on HepG2 cell line culture (**18)**. The samples with non-toxic concentration were checked for hepatoprotective activity on HepG2 cell line culture. 32mg/ml stocks of extracts were prepared using DMSO. Serial two fold dilutions were prepared from 20µg/ml to 0.312µg/ml using Dulbecco's Modified Eagle's Medium (DMEM) media for treatment. Stock cells of HepG2 cell line was cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100IU/ml), streptomycin (100µg/ml) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cell was dissociated with cell dissociating solution (0.2 % trypsin, 0.02 % EDTA, 0.05 % glucose in PBS). The viability of the cells are checked and centrifuged. Further, 50,000 cells/well were seeded in a 96 well plate and

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incubated for 24hrs at 37°C, 5% CO_2 incubator. The monolayer cell culture was trypsinized and the cell count was adjusted to 5 x 10^5 cells/ml using respective media containing 10% FBS. To each well of the 96 well microtiter plate, 100µl of the diluted cell suspension (50,000cells/well) was added. The plates were then incubated at 37°C for 24hrs in 5% CO_2 atmosphere. Paracetamol at the final concentration of 100µM in cell culture media was used to induce hepatotoxicity in HepG2 cell line. Paracetamol induced cells were incubated for 4hrs in a conventional incubator (37°C; 5% $C0_2$). After 4hrs, 100µl of different test concentrations of test samples were added on to the partial monolayer in microtiter plates. The plates were then incubated at 37°C for 24hrs in 5% CO_2 atmosphere. After incubation the media containing samples in the wells were discarded and 100µl of MTT (5mg/10ml of MTT in PBS) was added to each well. The plates were incubated for 4hrs at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 100µl of DMSO was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 590nm The percentage growth inhibition and proliferation was calculated using the following formulae.

% Inhibition = ((OD of Control – OD of sample)/OD of Control) x 100

III. RESULTS AND DISCUSSION

Preliminary phytochemical investigation

The medicinal plants contain some important types of biologically active compounds called phytochemicals which show potency towards the biological activity. Their confirmations, identifications, and characterization are very important. Preliminary phytochemical screening of leaves of Clerodendrum paniculatum revealed the presence of alkaloids, phytosterols, phenols, tannins, flavanoids and saponins (Table 1). The presence of terpenes, flavonoids, tannins, alkaloids, phenolic acid in the ethanolic extract of Clerodendrum paniculatum leaves were reported in earlier study (**19**). Many researchers reported that flavonoids and phenolic compounds in plants proved to possess multiple biological effects including antioxidant, anti-inflammatory, antimicrobial, antiangiogenic, anticancer, and antiallergic activities. In general, phenolic compounds and their derivatives are also considered as primary antioxidants or free radical scavengers (**20**).

Table 1. Qualitative phytochemical screening of various extracts of Clerodendrum paniculatum leaves

Sl. No.	Phytoconstituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	Alkaloids	-	+	-	-	-
2.	Carbohydrates	-	-	-	-	-
3.	Glycosides	-	-	-	-	-
4.	Phytosterols	+	-	-	-	-
5.	Phenolics and tannins	-	-	+	+	+
6.	Triterpenoids	-	-	-	-	-
7.	Flavanoids	-	-	+	+	+
8.	Saponins	-	-	-	+	+

"+": Indicates the presence and "–": Indicates the absence of phytochemicals

In-vitro evaluation of antioxidant activity

DPPH scavenging activity

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution (**21**). The DPPH scavenging activity of Clerodendrum paniculatum leaves extracts were tabulated (Table 2). Among all the extracts, the ethanolic extract showed 86.4% scavenging at 100µg concentration. The ethanolic extract exhibited a good potential to act as a free radical scavenger with IC_{50} of 55.2 µg for DPPH inhibition (Graph 1). This result could be due to the presence of flavonoid and phenolic compounds observed in the phytochemical screening of ethanolic extract of Clerodendrum paniculatum leaves. It has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties,

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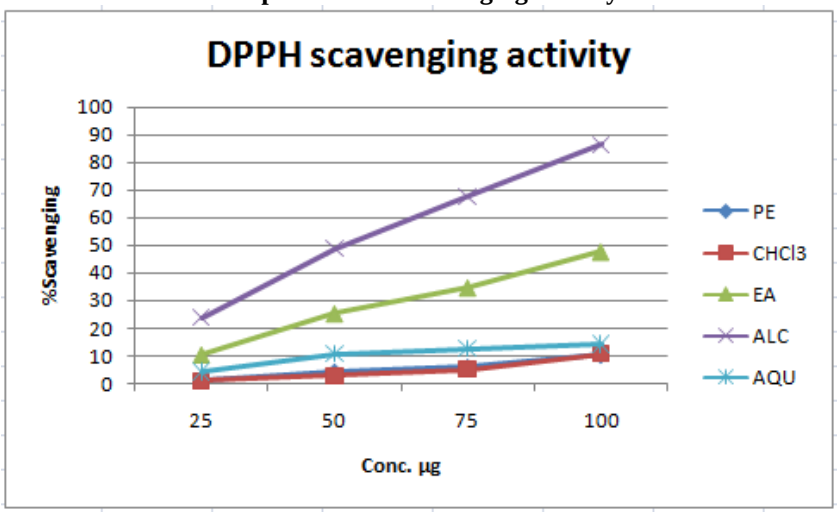
which can play an important role in adsorbing and neutralising free radicals (**22**).

Table 2. DPPH scavenging activity

Sl. No.	Extract	Concentration µg				IC 50 µg
		25	50	75	100	
		% Scavenging				
1.	PE	0.92	4.23	6.0	10.4	580
2.	CHCl3	1.25	2.85	5.4	10.85	614
3.	EA	10.3	25.3	34.7	47.8	105
4.	ALC	23.8	48.8	67.6	86.4	55.2
5.	AQU	4.3	10.9	12.7	14.4	591
6.	Ascorbic acid	96.4	-	-	-	

PE: Petroleum ether extract, CHCl3: Chloroform extract, EA: Ethyl acetate extract, ALC: Ethanol extract, AQU: Aqueous extract

Graph 1. DPPH scavenging activity



PE: Petroleum ether extract, CHCl3: Chloroform extract, EA: Ethyl acetate extract, ALC: Ethanol extract, AQU: Aqueous extract

Hydroxyl radical scavenging activity

Hydroxyl radicals are highly reactive and are short-lived. They are the major active oxygen species causing lipid peroxidation and enormous biological damage. They are capable of inducing detrimental effects on the important macromolecules including proteins and nucleic acids. The high reactivity of hydroxyl radicals lead to tremendous damage to the cell and its components and subsequently to the organisms as a whole. Several flavonoids synthesized by different plants as secondary metabolites have been reported to scavenge OH radicals earlier (**23**). In the present study the hydroxyl radical scavenging activity of the extracts of Clerodendrum paniculatum leaves was carried out is and the results were tabulated (Table 3). Among the extracts tested, the ethanolic extract showed better hydroxyl radical scavenging activity at 500mg/ml. The ethanolic extract showed the IC50 of 244.6 mg (Graph 2). The hydroxyl radical scavenging activity of the ethanolic extract may be due to the presence of phenolic compounds and flavonoids.

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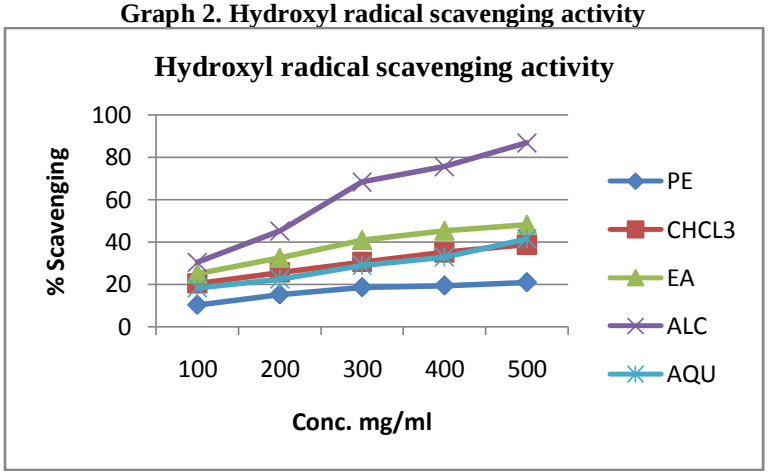
Table 3. Hydroxyl radical scavenging activity

Sl. No.	Extract	Concentration mg/ml					IC_{50} mg
		100	200	300	400	500	
		% Scavenging					
1.	PE	10.3	15.2	18.6	19.4	20.9	890
2.	CHCl3	20.4	25.6	30.5	35	38.7	499.3
3.	EA	25.1	32.5	40.9	45.3	48.2	390.6
4.	ALC	30.4	45.2	68.3	75.8	86.9	244.6
5.	AQU	18.3	22.4	28.9	32.8	41.5	521.2
6.	Ascorbic acid	96.7					

PE: Petroleum ether extract, CHCl3: Chloroform extract, EA: Ethyl acetate extract, ALC: Ethanol extract, AQU: Aqueous extract

Graph 2. Hydroxyl radical scavenging activity



PE: Petroleum ether extract, CHCl3: Chloroform extract, EA: Ethyl acetate extract, ALC: Ethanol extract, AQU: Aqueous extract

Nitric oxide scavenging activity:

Nitric oxide is a free radical produced in mammalian cells involved in regulation of various physiological processes. However, excess production of nitric oxide is associated with several diseases. The peroxynitrate anion is harmful to the cell activity which is formed when nitric oxide reacts with superoxide radical. Nitric oxide (NO) is a very unstable species because of its unpaired electron and is highly reactive to proteins and other molecules. The nitric oxide generated from sodium nitroprusside reacts with oxygen to produce stable nitrate and in the presence of antioxidant compounds, nitrite formation is in-hibited by directly competing with oxygen in the reaction with nitric oxide which is estimated by using Griess reagent at 546 nm **(24).** Among the different solvent extracts of Clerodendrum paniculatum leaves tested, the ethanolic extract showed good nitric oxide scavenging activity of 88.6% inhibition at 100µg with IC50 of 58.4 µg against the ascorbic acid with IC50 of 38.1 µg (Table 4 & Graph 3). The free radical scavenging property of the ethanolic extract of Clerodendrum paniculatum leaves are may be due to the presence of phenolic compounds and flavonoids.

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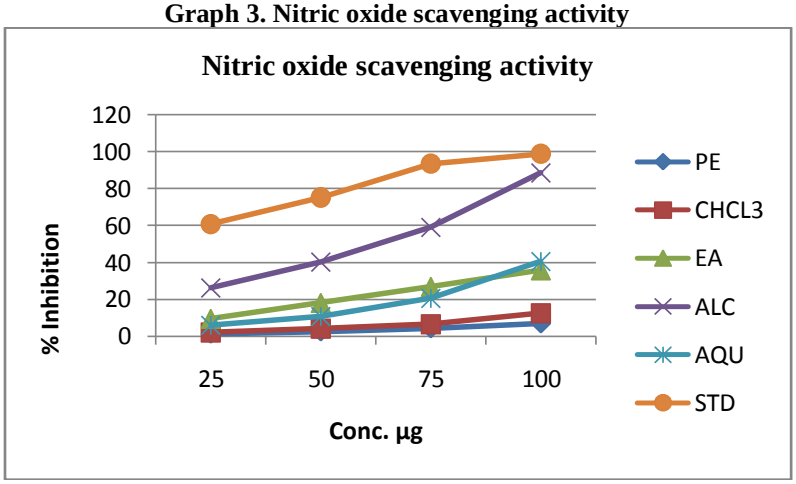
Table 4. Nitric oxide scavenging activity:

Sl. No.	Extract	Concentration µg				IC_{50} µg
		25	50	75	100	
		% Inhibition				
1.	PE	1.3	2.5	4.21	6.9	838.4
2.	CHCl3	2.1	4.25	6.45	12.5	494
3.	EA	9.6	18.2	26.9	35.8	138
4.	ALC	26.3	40.31	58.96	88.6	58.4
5.	AQU	5.96	10.79	20.58	40.5	160.6
6.	Ascorbic acid	60.8	75.2	93.4	98.8	38.1

PE: Petroleum ether extract, CHCl3: Chloroform extract, EA: Ethyl acetate extract, ALC: Ethanol extract, AQU: Aqueous extract

Graph 3. Nitric oxide scavenging activity



PE: Petroleum ether extract, CHCl3: Chloroform extract, EA: Ethyl acetate extract, ALC: Ethanol extract, AQU: Aqueous extract

Total Phenolic content

Phenolics possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression. Phenolics are the largest group of phytochemicals that are considered as primary antioxidants or free radical scavengers **(25)**.

It was thus reasonable to determine the phenolic content in the plant extract. The total phenolic contents of the ethanolic extract of Clerodendrum paniculatum leaves determined using the Folin-Ciocalteu reagent in comparison with standard gallic acid, and the result was expressed in terms of mg GAE/g. The results indicated that the total phenolic of ethanolic extract was found to be 138.2mg GAE/g (Table 5 & Graph 4).

Table 5. Total Phenolic content

Sl. No.	Gallic acid concentration µg/ml	Absorbance at 765nm			Total phenolic content (mg GAE/g)
		I	II	Avg	

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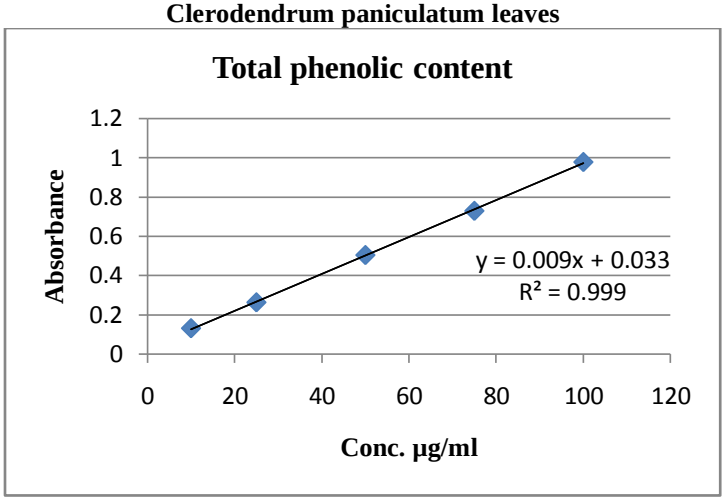
1.	10	0.133	0.135	0.132	138.2
2.	25	0.263	0.264	0.264	
3.	50	0.500	0.510	0.505	
4.	75	0.732	0.729	0.730	
5.	100	0.977	0.982	0.979	
Extract	1000	1.276	1.279	1.277	

Graph 4. Estimation of total phenolic (Gallic acid equivalents) in ethanolic extract of Clerodendrum paniculatum leaves



Total Flavonoid content

Flavonoids are the largest group of naturally occurring phenolic compounds, which occurs in different plant parts both in free state and as glycosides. Flavonoids are particularly beneficial, acting as antioxidants and giving protection against cardiovascular disease, certain forms of cancer and age-related degeneration of cell components. Their polyphenolic nature enables them to scavenge injurious free radicals such as super oxide and hydroxyl radicals **(23).** The total flavonoids of the ethanolic extract of Clerodendrum paniculatum leaves was determined by Aluminum chloride colorimetric method and expressed as mg quercetin equivalent per gram of the extract. The total flavonoids of the ethanolic extract of Clerodendrum paniculatum leaves was found to be 32.6mg QE/g (Table 6 & Graph 5).

Table 6. Total Flavonoid content

Sl. No.	Quercetin concentration µg/ml	Absorbance at 415nm			Total flavonoid content (mg QE/g)
		I	II	Avg	
1.	10	0.158	0.165	0.161	32.6
2.	20	0.370	0.368	0.369	
3.	40	0.745	0.761	0.753	
4.	60	1.096	0.916	1.006	
5.	80	1.325	1.305	1.315	
6.	100	1.615	1.595	1.605	
Extract	500	0.550	0.560	0.540	

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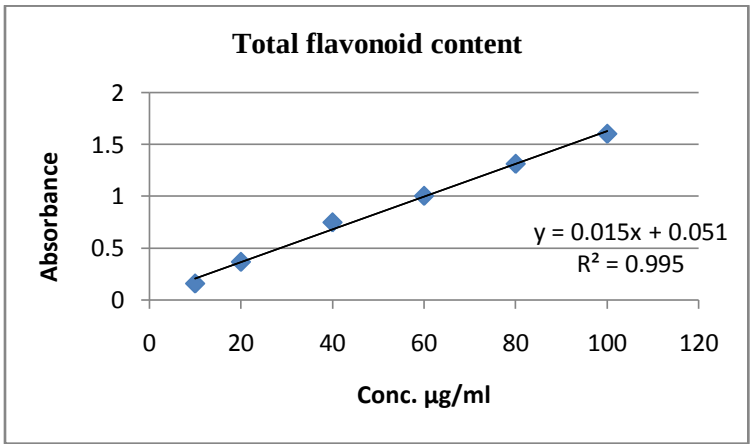
Graph 5. Estimation of total flavonoids (Quercetin equivalents) in ethanolic extract Clerodendrum paniculatum leaves



Cytotoxicity study (MTT assay)

The MTT assay has been most widely applied in the assessment of cytotoxic drug therapy. This assay relies on the cellular reduction of tetrazolium salts to their formazan crystals. The range of cell concentrations in which there is a direct relationship between optical density and the number of cells counted should be determined. Towards the end of the incubation period for a given assay, the MTT solution is added and the plate is incubated for an additional time period. The formazan crystals are then dissolved in an acid/alcohol solution and the optical density is determined. Optical density values of the treated cells are compared with the optical density values of the control cells and results are presented as a percentage of cell survival. Cell number is then calculated based on the calibration curve (**26,27).** The sample concentrations to be tested for in vitro hepatoprotective were selected based on MTT assay with Doxorubicin induced cytotoxicity**.** Doxorubicin (DOX) is a cytotoxic anthracycline (**28)** which is used as control. The cells treated with Doxorubicin with 100µM showed complete cytotoxicity with IC50 value as 19.58 µM. At the concentration of 320µg/ml the ethanolic extract of C. paniculatum showed 42.18% cell growth inhibition which indicates the less cytotoxicity of ethanolic extract than the other extracts (Table 7). The less morphological changes in the HepG2 cells treated with 10 µg/ml and 320 µg/ml concentrations of extracts was observed with ethanolic extract of C. paniculatum.(Fig.1h)

Table 7. Cytotoxicity studies (MTT assay) of extracts of Clerodendrum paniculatum leaves:

	% inhibition at 590nm				
Extracts / Conc. µg/ml	**CPP**	**CPC**	**CPEA**	**CPE**	**CPW**
10	22.86	15.64	14.45	2.89	15.77
20	29.57	22.60	21.68	8.15	25.36
40	39.82	35.74	29.83	19.58	31.14
80	45.60	44.81	37.84	29.83	39.16
160	64.78	57.29	52.69	37.58	55.32
320	73.46	69.25	59.92	42.18	60.71
IC50 µg/ml	112.97	76.18	88.55	--	93.02

CPP- Clerodendrum paniculatum petroleum ether extract, CPEA- Clerodendrum paniculatum ethyl acetate extract, CPC- Clerodendrum paniculatum chloroform extract, CPE- Clerodendrum paniculatum ethanolic extract, CPW- Clerodendrum paniculatum aqueous extract.

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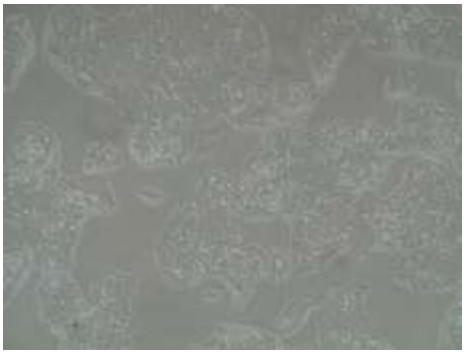
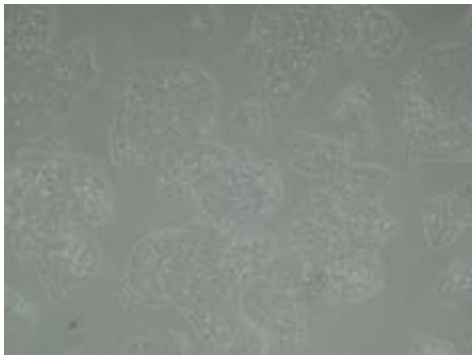
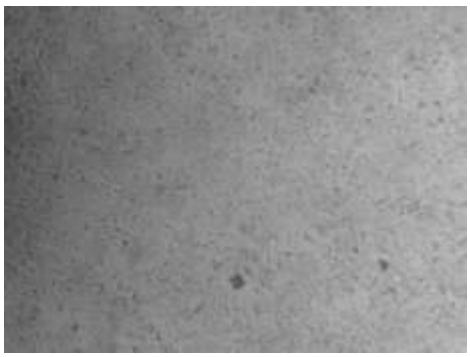
Fig 1: Cytotoxicity study (MTT assay) of Clerodendrum paniculatum leaves extracts.



Control



1a



1b

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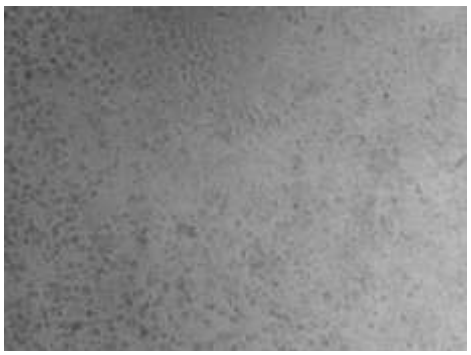

1d

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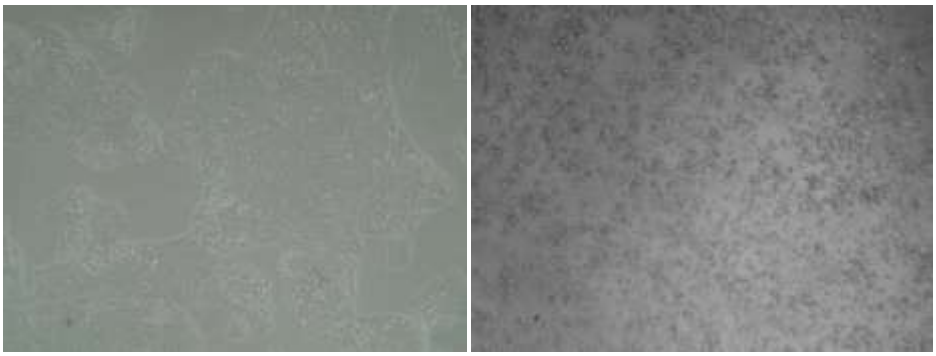
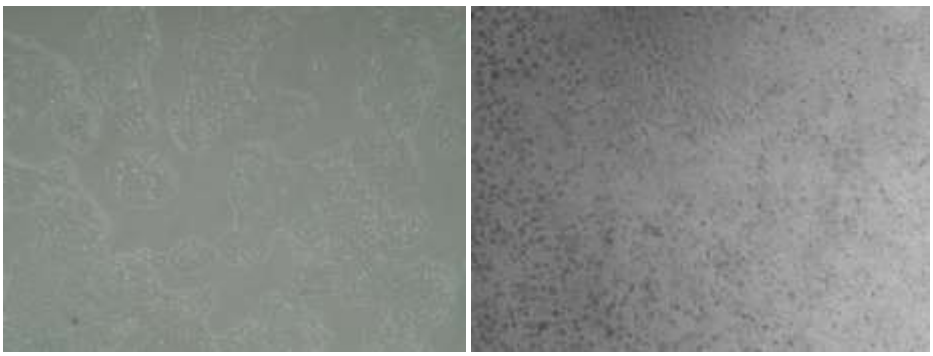


1e 1f

1g 1h



1i 1j

Fig.1a CPP- Clerodendrum paniculatum petroleum ether extract 10 µg/ml, 1b CPP- 320 µg/ml, 1c CPC- Clerodendrum paniculatum chloroform extract -10 µg/ml, 1d CPC- 320 µg/ml, 1e CPEA- Clerodendrum paniculatum ethyl acetate extract -10 µg/ml, 1f CPEA- 320 µg/ml, 1g CPE- Clerodendrum paniculatum ethanolic extract -10 µg/ml , 1h CPE- 320 µg/ml, 1i CPW- Clerodendrum paniculatum aqueous extract -10 µg/ml, 1j CPW- 320 µg/ml

Hepatoprotective activity

The hepatoprotective activity of the extracts were tested by MTT assay [(3-(4,5 dimethylthiazole –2 yl)-2,5 diphenyl tetrazolium bromide) assay]. The principle involved is the cleavage of tetrazolium salt MTT into a blue coloured derivative by living cells. The tetrazolium salt (3-(4, 5 dimethylthiazole –2 yl)-2,5 diphenyl tetrazolium bromide) is taken up into the cells and reduced in a mitochondria dependent reaction to yield a blue coloured formazan product. The product accumulates within the cell, due to the fact that it cannot pass through the plasma membrane. On solubilisation of the cells, the product is librated and can be readily detected and quantified by a simple colorimeric method (**29)**.

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The HepG2 hepatoma cell line is used widely in studies of liver function, metabolism, and drug toxicity. These cell lines have been proposed as an alternative to human hepatocytes for in vitro models of normal liver cells. The potential advantages of hepatoma cells are that, as an immortalized cell line, they are readily available in large quantities, they are easy to maintain because they can be cryopreserved, and their drug-metabolizing enzyme activities do not decrease in cultivation, as happens in primary cultures of human hepatocytes. So these cells are used in studies to determine the medicinal plants for hepatoprotective activities (**30,31)**. HepG2 cell line has also been extensively exploited to examine cytoprotective, antioxidative, hepatoprotective, anti-hepatoma, hypocholesterolemic, anti-steatosis, bioenergetic homeostatic and anti-insulin resistant properties (**32)**. The percentage hepatoprotective activity was determined with respect to the Paracetamol induced hepatotoxicity. Paracetamol at 100µM concentration showed 51.08% cytotoxicity in HepG2 cells. Ethanolic extracts of C. paniculatum showed 41.77% of cell viability respectively at 20µg/ml in HepG2 cells against Paracetamol induced toxicity which was better than the other extracts at 20µg/ml. Standard Silimyrin showed hepatoprotective effect of 66.58% at 12.5µg/ml in HepG2 cells. (Table 8 & Graph 6). The possible mechanism of hepatoprotective activity of ethanolic extracts of C. paniculatum may be due to the presence of flavonoids in the extracts. Even the free radical-scavenging activity of the C. paniculatum extracts evaluated by DPPH assay revealed that the ethanolic extract showed good antioxidant effect. The phytochemical screening of Clerodendrum paniculatum showed the presence of flavonoids. Flavonoids, a vast class of hydroxylated phenolic substances, have long been reported as antioxidants in plants, which is due to their ability to scavenge free radicals and to reduce free radical formation. Recent research in the field of free radical biology suggested an important pathophysiological role of free radicals and oxidative stress in the development and progression of liver diseases. Hence, antioxidants are frequently used to treat oxidative liver injury, and the consumption of antioxidants is known to be an important means of preventing or delaying the appearance of liver diseases **(33).** The antioxidant activity of flavonoids can scavenge free radical and protect the cell membrane from destruction.

Table 8. In-vitro hepatoprotective activity of Clerodendrum paniculatum leaves extracts.

	% inhibition at 590nm					
Extracts / **Conc. µg/ml**	**CPP**	**CPC**	**CPEA**	**CPE**	**CPW**	**Silimyrin Conc. 12.5 µg/ml**
0.3125	60.89	51.96	51.95	52.42	55.34	66.58
0.625	63.19	53.18	53.16	53.63	56.43	
1.25	63.60	53.72	53.71	54.17	58.05	
2.5	65.22	55.48	55.33	55.93	60.08	
5	68.47	56.70	56.55	55.80	63.87	
10	70.23	60.89	67.51	57.29	65.76	
20	72.12	69.42	70.62	58.23	70.09	

CPP- Clerodendrum paniculatum petroleum ether extract, CPC- Clerodendrum paniculatum chloroform extract, CPEA- Clerodendrum paniculatum ethyl acetate extract, CPE- Clerodendrum paniculatum ethanolic extract, CPW- Clerodendrum paniculatum aqueous extract.

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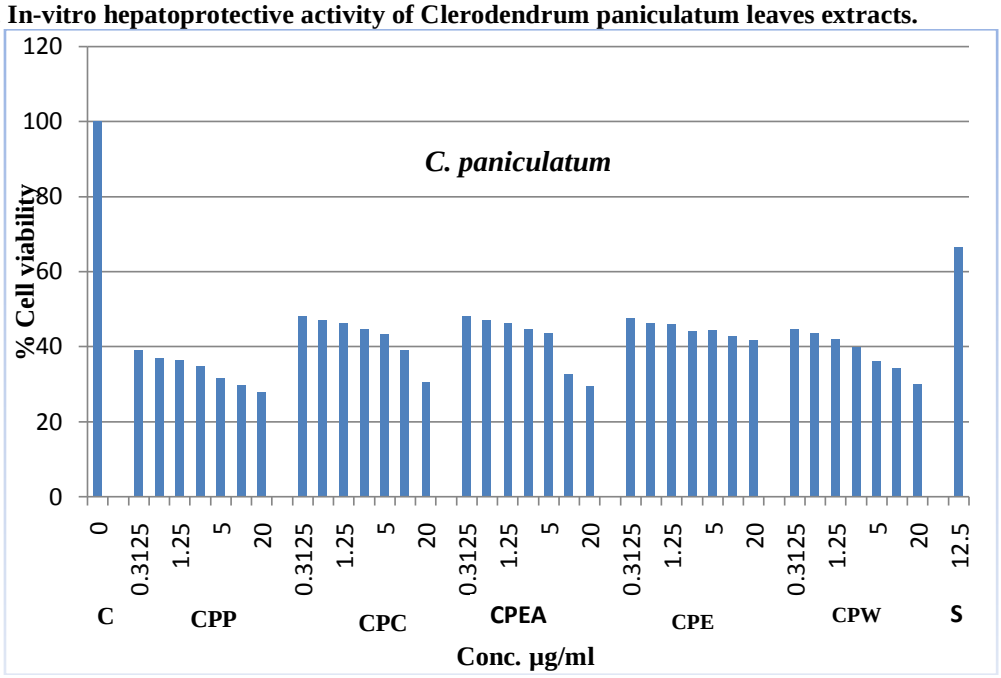
Graph 6. In-vitro hepatoprotective activity of Clerodendrum paniculatum leaves extracts.



C- Control, CPP- Clerodendrum paniculatum petroleum ether extract, CPC- C. paniculatum chloroform extract, CPEA- C. paniculatum ethyl acetate extract, CPE- C.paniculatum ethanolic extract, CPW- C. paniculatum aqueous extract, S- Silymarin

IV. CONCLUSION

Preliminary phytochemical screening of leaves of Clerodendrum paniculatum revealed the presence of alkaloids, phytosterols, phenols, tannins, flavanoids and saponins. The better antioxidant activity of the ethanolic extract of Clerodendrum paniculatum leaves may be due to the presence of flavonoid and phenolic compounds. Even the ethanolic extract of Clerodendrum paniculatum leaves showed better in vitro hepatoprotective activity in Paracetamol induced hepatotoxixity in HepG2 cell line culture using Silymarin as standard. The hepatoprotective activity of the ethanolic extract of Clerodendrum paniculatum leaves may be attributed to its antioxidant properties.

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