IJPSR (2024), Volume 15, Issue 5 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 November 2023; received in revised form, 04 January 2024; accepted, 05 April 2024; published 01 May 2024

PHYTOCHEMICAL SCREENING, ANTIOXIDANT AND *IN-VITRO* ANTICANCER ACTIVITIES OF *TINOSPORA CORDIFOLIA* LEAVES EXTRACTS

Pradeep Kumar Samal *, Mohanish Sharma, Bharti Vaishnaw, Aarti Tiwari, Kamdev Sen, Dipendra Nirmalkar and Pameshwar Kumar

Department of Pharmacy, Guru Ghasidas Vishwavidyalaya, Bilaspur - 495009, Chhattisgarh, India.

Keywords:

Tinospora cordifolia, Ethanolic extracts, Cyclophosphamide, 5-Fluorouracil, Micronucleus

Correspondence to Author:
Pradeep Kumar Samal

Research Scholar,
Department of Pharmacy,
Guru Ghasidas Vishwavidyalaya,
Bilaspur - 495009, Chhattisgarh,
India.

E-mail: samalpharmacology@gmail.com

ABSTRACT: Globally, cancer is a disease that is affecting the human population in abundance. According to the traditionally ayurvedic literature, *Tinospora cordifolia* are commonly applied in the treatment of various diseases. The present study was designed to investigate the antioxidant and anticancer activities of *Tinospora cordifolia* on the basis of its on biochemical and histopathological parameters. These included estimations of lipid peroxidation, CAT activity, GSH level, micronucleus assay and histopathological studies. In this study, phenolic and flavonoid content was found in leaves of *Tinospora cordifolia*. Cyclophosphamide (75 mg/kg; i.p.) is anticancer drug but it was used to induce dominant lethal mutation, micronuclei, DNA damage and generation of reactive oxygen species (ROS) in bone marrow for the estimation of antioxidant and anticancer activity. 5-Fluorouracil (30 mg/kg; i.p.) used as a standard, and ethanolic and aqueous extract of *Tinospora cordifolia* (250mg/kg, 500 mg/kg, 1000mg/kg; p.o.) is used as a preventive therapy. This study concluded that, both extract of *T. cordifolia* exerts antioxidant and anticancer activity.

INTRODUCTION: Our healthcare system will continue to face challenges from cancer, which will be responsible for around 10 million deaths in 2020, or almost one in every six [1]. Cancer is a disease in which the rhythm of tissue growth gets disturbed. To create this situation of tissue, that gene is responsible which monitors the life cycle of the cell. Unrestricted cell proliferation and malfunctioning replicative senescence processes are typical characteristics of the disease states [2]. Chemotherapy plays a special role in cancer treatment, but its side effects are also such that the patient has to go through many stressful situations and health damaging problems.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(5).1485-96

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(5).1485-96

That's why our focus is towards natural compound which shows minimum side effects because herbal medicines have fewer adverse effects and are more suited to the human body, about 70–80% of individuals continue to utilise them for their primary health. The fruits and vegetables included in our diet are rich in antioxidants. This antioxidant reduces the risk of many cancer diseases and prevents cancer from spreading [3]. Natural compounds such as polyphenolic compound, flavonoid compound, tannins and organosulfur all consider to be anticancer compound [4].

We can see as an example in some research papers; Formononetin in the treatment of gastric cancer, curcumin useful for colon, gastric, breast, lung, head and neck cancer, hesperidin for non-small-cell lung carcinoma treatment, Epigallocatechin gallate uses for skin cancer, quercetin involve in the treatment of colorectal and breast cancer, allicin in Ovarian cancer and all this plants also have

antioxidant property [5-12]. *T. cordifolia* is also known as Guduchi and its names in Latin: *Tinospora cordifolia*, Hindi: Giloya, it belongs to the family of Menispermaceae [13]. The chemical constituents of *T. cordifolia* belong to different classes such as alkaloids, glycosides, steroids, aliphatic compounds, polysaccharides, leaves are rich in protein, calcium and phosphorus [14]. They estimated the total flavanol and total phenolic content. Based on the result of the formulation showed potent antioxidant activity. So, seeing the possible correlation between Antioxidant and Anticancer activities, I have conducted the study regarding the Antioxidant and Anticancer activity of *Tinospora cordifolia*.

MATERIALS AND METHODS:

Plant Material: Fresh leaves of *Tinospora cordifolia* were collected from Thakur Chhedilal Barrister Agriculture College and Research Centre, Bilaspur, Chhattisgarh in month of September 2012. The plant material has been authenticated by Dr. H. B. Singh, Director, Museum of Specimens and Raw Materials, National Institute of Science, Information Resources and Communication (NISCAIR), New Delhi. The specimen voucher no. (NISCAIR/RHMD/ Consult/-2012-13/2120/127) was deposited in the dept. The crude drug was powdered by hand grinder after drying.

Drug and Chemicals: Ethanol (CDH, Mumbai), bovine serum albumin, Thiobarbituric acid (TBA), Sodium azide, Nitro blue tetrazolium (NBT), NADH, 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB), Phenazine methosulphate were procured from HIMEDIA, Mumbai, India. Benzene, Ethyl acetate and other phytochemical reagents were provided by Institute. 5-Flucel (5-Fluoro uracil), Endoxan-500 (Cyclophosphamide) and was purchased from local market.

Preparation of Extract: The powdered drug was taken and subjected for successive solvent extraction.

Preparations of Benzene Extract: *Tinospora cordifolia* leaves washing with tape aqueous and air dried at room temperature. About 200 g of the leaves powder was extracted with 1.0 L of Chloroform using Soxhlet apparatus for 72 hrs at 70-80°C. The extract is concentrated to one-fourth of the original volume by distillation because it is suitable for recovering the solvent, which can be reused for extraction.

Preparation of Ethyl Acetate Extract: After complete drying of, the above marc remained after Benzene extraction was extracted with Ethyl acetate to get Ethyl acetate extract. Similarly, if in place of ethyl acetate if we use ethanol and aqueous, then we will obtain ethanolic and aqueous extract preparation respectively.

FIG. 1: *TINOSPORA CORDIFOLIA*

Preliminary Phytochemical Screening: After receiving the extract, the help of many test methods was taken to find out the different chemical components present in it [15-17].

Experimental Animals: Wistar albino rats weighing 150–200 grammes were given by the pharmacy department and kept in the department's animal house for research purposes. Then all the animals were acclimatized for seven days under standard husbandry conditions, i.e.; room temperature of 25±1°C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet (Pranav Agro Industries Ltd, Vadodara, India), with aqueous supplied *ad libitum* under strict hygienic conditions. Each experimental group had separate set of animals and care was taken to ensure that animals used for one response were not employed elsewhere. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. Prior to the experiments, the SLT Institute of Pharmaceutical Sciences, Bilaspur, Chhattisgarh's Institutional Animal Ethical Committee (IAEC) gave its consent. All the protocols and the experiments were conducted in strict compliance according to Institutional Animal ethical Committee and guidelines (Reference No.

24/IAEC/Pharmacy/ 2013) provided by committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Approval No.994/a/GO/ 06/CPCSEA).

Determinations of Acute Oral Toxicity (LD_{50}): The acute oral toxicity (AOT) of extract of leaves of *Tinospora cordifolia* was determined according to OECD guideline no. 423. Female albino mice weighing between 25-30 g, maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment. Study has been performed in two phases, first and second. Twelve animals divided into four groups (G-I to G-IV) having three in each group. In first phase a single dose of 300 mg/kg, 500 mg/kg and 1000mg/kg dissolved in 1% v/v Tween-40 were administered to Animals G-I to G-III and observed for mortality up to 24 hours (short term toxicity). Based on short-term profile of drug, again a single dose of 2000mg/kg was administered to the G-IV and animals were observed for mortality and clinical sign of toxicity (14 days) [18].

Pharmacological Activity in Extract of *Tinospora cordifolia* Leaves:

Evaluation of Anti-mutagenic and Antioxidant Activity: The experimental protocol is in accordance with the OECD guideline for mutagen city studies in animals. Fifty four animals were divided into 9 groups (Group I-IX) of six animals each: Group I - Treatedas Normal control, which were received distilled aqueous (10ml/kg; by gavage,) for 7 days, Group II – Treated as Control, which were received a single dose of Cyclophosphamide (75 mg/kg; i.p.) in saline aqueous, 1 hr after the last dose of distilled aqueous (10ml/kg; by gavage) for 7 days, Group III - Treated as Standard, which were received 5-FU (30 mg/kg; i.p.), for 7 days and Cyclophosphamide (75 mg/kg; i.p.), 1 hr after the last dose of 5-FU, Group IV - Treated as Test 1, which were received a single dose of cyclophosphamide (75 mg/kg; i.p.),1 hr after the last dose of Ethanolic extract (250 mg/kg, for 7 days) in 1% Tween-40 (10ml/kg by gavage), Group V - Treated as Test II which were received a single dose of Cyclophosphamide (75 mg/kg; i.p.),1 hr after the last dose of Ethanolic extract (500 mg/kg, for 7 days) in 1% Tween-40 (10ml/kg; by gavage), Group VI - Treated as Test III which were received a single dose of Cyclophosphamide (75 mg/kg; i.p.), 1 hr after the last dose of Ethanolic extract (1000 mg/kg, for 7 days) in 1% Tween-40 (10ml/kg; by gavage), Group VII - Treated as Test IV which were received a single dose of Cyclophosphamide (75 mg/kg; i.p.), 1 hr after the last dose of Aqueous extract (250 mg/kg, for 7 days) in 1% Tween-40 (10ml/kg; by gavage), Group VIII- Treated as Test V which were received a single dose of Cyclophosphamide (75 mg/kg; i.p.), 1 hr after the last dose of Aqueous extract (500 mg/kg, for 7 days) in 1% Tween-40 (10ml/kg; by gavage, Group IX- Treated as Test VI which were received a single dose of Cyclophosphamide (75 mg/kg; i.p.), 1 h after the last dose of Aqueous extract (1000 mg/kg, for 7 days) in 1% Tween-40 (10ml/kg; by gavage).

Micronuclei Determination:

Cleaning of Glass Slides: A saturated solution of potassium dichromate in concentrated sulphuric acid was made and stored. The solution was diluted with aqueous for cleaning. The glass slides (Blue Star-Super deluxe, Mumbai) were soaked overnight in this solution and cleaned with distilled aqueous next day. The slides were air dried and used to prepare smear.

Preparation of Phosphate Buffer Solution (PH-6.8): 2.366 g of disodium hydrogen phosphate was weighed and dissolved in 250 ml of distilled aqueous - Solution A. 2.27 g of potassium dihydrogen phosphate was weighed and dissolved in 250 ml of distilled aqueous - Solution B. 50 ml of Solution A and Solution B were mixed and volume was made up to 1000 ml with distilled aqueous.

Preparation of Suspending Medium: 5 % bovine albumin solution was prepared by dissolving the required quantity of bovine albumin powder in saline solution (0.9% NaCl) having pH-7.2. Two drops of 1 % sodium azide were added as a preservative.

Preparation of Staining Solutions: May-Grunwald's stain was prepared by dissolving 0.2 g of the stain powder in 100 ml of methanol with slight heating and stirring. Once it dissolved completely, it was filtered. Giemsa's stain was prepared by dissolving 1 g of Giemsa's stain in 54

ml of glycerine. It was kept at 600 °C for 2 hrs, after cooling 84 ml of methanol was added, stirred well and filtered.

Extraction of Bone Marrow and Preparation of Smear: After mice were treated with Cyclophosphamide for 30 hrs, mice were sacrificed by cervical dislocation. Femur and tibia were removed from animals after they had been cut open, and the upper end of the femur was cut open until a tiny opening was evident. A 21-gauge needle using a disposable syringe was inserted to ensure that the upper end was open. A syringe containing roughly 0.5 ml of the suspending medium was filled, and a needle was then inserted at the lower epiphysial end. A clear cavity block was used to flush the marrow.

If the marrow was solid, it was flushed and aspirated repeatedly with the aid of the syringe to disseminate it. Similarly, tibial marrow was also collected. The marrow from the femur and tibia could be collected using a total of 2 ml of the suspending medium. The contents of the cavity block were carefully combined to create a fine marrow suspension. The suspension was then transferred to a centrifuge tube and centrifuged at 1000 rpm for 5 min. the supernatant was drawn off and marrow films made by smearing a small drop on a clean glass slide, then the slides were air-dried. For each mouse, four images were created [19].

Staining Procedure: For 10 minutes, the smears were fixed in pure methanol. For 15 minutes, the slides were kept in coupling jars with newly diluted May-stain Grunwald's and an equal amount of phosphate buffer (PH-6.8). The slides were then transferred to coupling jars containing Giemsa's stain freshly diluted with phosphate buffer (1:6) and kept for 10 min. Slides were quickly cleaned in three or four changes of buffered aqueous. After that, the transparencies were left in buffered aqueous for 5 minutes without being touched so that differentiation could occur. The slides were finally air dried.

Scoring: Scoring the nucleated BMCs and the percentage of micronucleated Bone marrow cells (MN-BMCs); mono, bi- and polynucleated MN-BMCs, was determined by analyzing their number in 1000 BMCs per rat.

Biochemical Estimation of Antioxidantenzymes: After collection of bone marrow, the rats were dissected and their liver was excised. The separated liver was rinsed with ice-cold normal saline followed by a cold phosphate buffer with a pH of 7.4, then dried and weighed. A 10% w/v homogenate of liver was prepared in ice cold phosphate buffer (0.1 M, pH 7.4) and a portion were utilized for estimation of lipid peroxidation and other portion of the same after precipitation of proteins with TCA was used for estimation of glutathione, remaining homogenate were centrifuged at 4000 rpmat 4°C for 5 min. Superoxide dismutase, catalase, and protein content were estimated using the supernatant that was subsequently collected.

Estimation of lipid Peroxidation: Malondialdehyde (MDA), a measure of lipid peroxidation, was assayed in the form of Thiobarbituric acid reactive substances (TBARS). In a nutshell, 0.8% Tiobarbituric acid, 1.5 ml of 20% acetic acid, and 0.2% of sodium dodecyl sulphate were sequentially added to 0.2% of tissue homogenate. The volume of mixture was made up to 4 ml with distilled aqueous. The mixture was incubated for 60 min. at 95°C in a temperature control aqueous bath and cooled and added 5 ml of n-butanol: pyridine (15:1) mixture was added and the contents were vortexed thoroughly for 2 min. The top organic layer was separated after 10 minutes of centrifugation at 3000 rpm, and absorbance was measured at 532 nm in comparison to a suitable blank devoid of the sample. The levels of lipid peroxides were expressed as nanomoles of Thiobarbituric acid reactive substances (TBARS)/mg protein using an extinction coefficient of $1.56\ 10^5\ M^{-1}\ cm^{-1}$.

Catalase Activity (CAT): The catalase activity was determined in liver homogenate by using Aebi's (1984) method. After the centrifugation of liver supernatant 0.05 ml was added to a test tube containing 2 ml of Phosphate buffer (pH -7.0) and 1 ml of 30 mM H_2O_2 and mixed well. Catalase activity was measured at 320 nm for 1 min in the time interval of 10 sec against blank using spectrophotometer. The catalase activity was calculated using the 43.6 M cm^{-1} molar extinction index of H_2O_2. One unit of activity is equal to one

millimoles of H_2O_2 degraded per minute and is expressed as unit per milligram of tissues.

Estimation of Reduced Glutathione (GSH): Reduced glutathione was estimated spectrophotometerically by determination of dithiobis-(2-nitrobenzoic acid; DTNB) reduced by SH-groups and expressed as mole/mg of protein [20]. To 0.1 ml of different tissue samples, 2.4ml of 0.02M EDTA solution was added and kept on ice bath for 10 minutes. Then 2 ml of distilled aqueous and 0.5 ml of 50% w/v TCA were added. This mixture was kept on ice for 10-15 minutes and then was centrifuged at 3000x g for 15 minutes. To 1ml of supernatant, 2.0ml of Tris buffer (pH 0.8) was added. After that, 0.05 ml of the DTNB solution (Ellman's reagent) was introduced and vigorously vortexed. OD was read (within 2-3 minutes after the addition of DTNB) at 412 nm in spectrophotometer against a reagent blank. Appropriate standards were run simultaneously.

Total Phenolic Content: The total phenolic content was determined by adding 0.5 ml of extract aqueous solution to 2.5 ml, 10% Folin-Ciocalteu reagent (v/v) and 2.0 ml of 7.5% Na_2CO_3. The reaction mixture was incubated at 45 $^{\circ}$C for 40 min, and the absorbance was measured at 765nm in Schimadzu spec-1800, spectrophotometer. Gallic acid was used as a standard phenol (10-50 µg/ml), giving the calibration equation: $y = 0.0185x$ (R^2 = 0.9901), where x is the gallic acid concentration in µg/ml and y is the absorbance reading at 765 nm. The findings of each test were performed in triplicate and expressed as gallic acid equivalents (mg GAE/g dry weight of the ORE) [21].

Total flavonoid Content: The flavonoid content was determined based on the formation of flavonoid–aluminium complex. One millilitre of sample was mixed with 1ml of 2% aluminium chloride solution. After incubation for 15 min at room temperature, the absorbance of the reaction mixture was measured at 306 nm. A standard curve was first plotted using gallic acid (10-50 µg/ml) as a standard, giving the calibration equation:

$$Y = 0.0078 \text{ x } (R^2 = 0.9281)$$

Where, x is the concentration of gallic in µg/ml, and y is the absorbance reading at 306 nm. The amount of flavonoids was expressed as gallic acid equivalents (mg GAE/g dry weight) and all tests were carried out in triplicate [22].

Statistical Analysis: The collected data were subjected to appropriate statistical test like one-way ANOVA (Analysis of variance), followed by an appropriate turkey test. P values of less than 0.01 were considered as significant. The analysis was carried out using Graph pad prism software of version 5.

HPTLC Analysis of Extracts: The HPTLC system (CAMAG, Muttanz, Switzerland) consisted of (i) TLC scanner connected to a PC running WinCATS software under MS DOS. (ii) Linomat IV automatic sample applicator; CAMAG (Muttenz, Switzerland) using 100µl syringe and connected to a Nitrogen tank. (iii) TLC Chamber: Glass twin trough chamber ($20\times10\times4cm^3$); CAMAG. (iv) HPTLC plates: 20×10 cm^2, 0.2mm thickness precoated with silica gel 60 F254; E. Merck (Darmstadt, Germany). (v) Experimental conditions: temperature 25 ± 2^0C, relative humidity 40% and Solvent system: toluene: ethyl acetate (9:3, v/v).

Organ Collection and Histopathological Studies: At theends of 10^{th} day study. The animals were sacrificed; liver was washed with normal saline and preserved in 10% formalin for histopathological studies. Liver tissues were embedded in liquid paraffin, cut in to 5-6 µm and stained with haemotoxylin and eosin for histopathological findings.

RESULTS:

Phytochemical Results:

TABLE 1: PHYSICAL APPEARANCE AND EXTRACTIVE VALUES OF LEAVES OF *TINOSPORA CORDIFOLIA*

Drug	Solvent	Colour	%Extractive value
Tinospora cordifolia	Benzene Ethyl acetate	Dark green Greenish black	22.2
Ethanol Black AqueousDark brown	17.2	20.5	18.0

TABLE 2: PRELIMINARY PHYTOCHEMICAL SCREENING OF PLANT EXTRACTS

S. no.	Test	Extracts			
		Benzene	Ethyl acetate	Ethanol	Aqueous
1.	Alkaloids				
	Wagner's test	+	-	+	+
	Dragendroff s test	-	-	-	+
	Hagers test	-	-	+	-
2.	Glycoside				
	Legals test	-	-	+	+
	Killer killani test	-	-	-	+
3.	Saponins				
	Froth test	-	-	+	+
	Liberman Buurchard test	-	-	+	+
4.	Phytosterols				
	Salkowski's test	-	-	+	-
	Lliberman's Burchard test	-	-	+	-
5.	Phenolics And Tannins				
	Ferric chlorides test	-	-	+	+
	Lead acetate test	-	+	+	+
	Shinoda test	-	-	+	+
6.	Proteins And Amino Acids				
	Millons test	-	-	-	-
	Biuret test	-	-	-	-
	Ninhydrin test	-	-	-	-
7.	Fixed Oils And Fats				
	Stain test	-	-	-	-
	Soap test	-	-	-	-
8.	Carbohydrates				
	Molish test	-	-	+	+
	Benedicts test	-	-	-	+
	Fehling's test	-	-	+	+

The (+) sign indicate presence of compound and (-) sign indicate the absence of compound.

Acute oral Toxicity Study: Different doses of Benzene, Ethyl acetate, Ethanolic and aqueous extracts were screened for their oral toxicity. No mortality was recorded till 2000 mg/kg with all extracts; hence the extracts were found to be safe up to the dose levels of 2000 mg/kg.

Total Phenolic Content: The Folin–Ciocalteu's assay is a fast and simple method for rapidly determined phenolic contents in samples [23]. The ethanolic extract (2.5 mg/ml) having the total phenolic content of (13.6mg ± 0.79 mg GAE/g) exhibited high total phenolic content, which appears higher than that the aqueous extract (5mg/ml) of *Tinospora cordifolia* (3.08±0.51mg GAE/g).

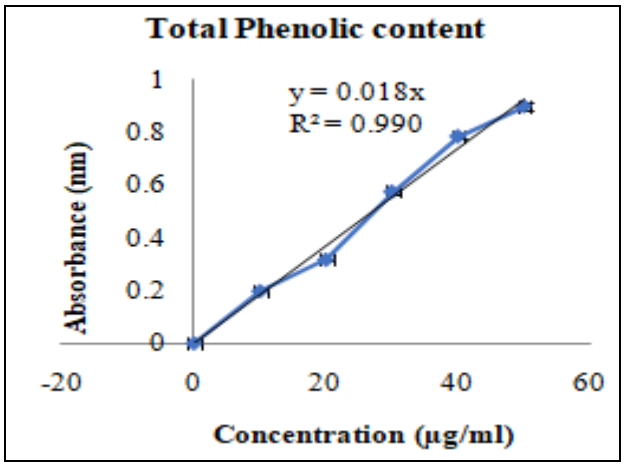

FIG. 2: GRAPH SHOWING STANDARD CURVE OF TOTAL PHENOLIC CONTENT

Total Flavonoids Content: The total flavonoids content in extracts was determined based on the formation of flavonoid–aluminium complex [22]. The amount of total flavonoids assessed in ethanolic extract (0.2mg/ml) was 97.5±0.67 mg GAE/g, on the other hand the total flavonoids assessed in aqueous extract (1mg/ml) was found to be 44.87±0.55 mg GAE/g. This content appears higher then that the flavonoids content detected in *Opuntia ficus indica* (L.) Mill fruit juice (652.5±38 µg/ml), known for their antioxidant and antiulcerogenic activities [24].

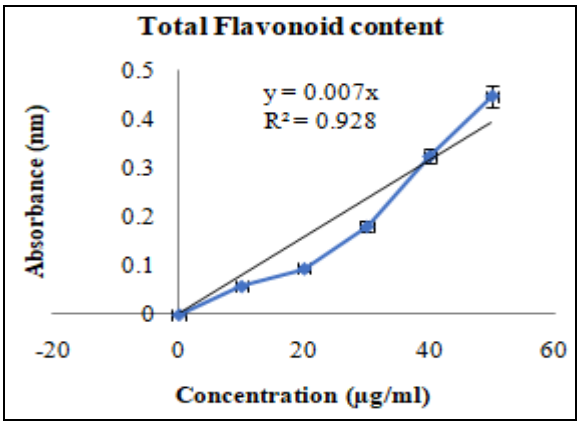

FIG. 3: GRAPH SHOWING STANDARD CURVE OF TOTAL FLAVONOID CURVE

Antioxidant Activity of Ethanolic and Aqueous Extracts of *T. Cordifolia*:

Catalase (CAT): CAT activity was significantly (P <0.001) reduced in Cyclophosphamide (CP) treatment when compared to Normal control. The ethanolic extract of *T. cordifolia* (EETC) (250 mg/kg, 500 mg/kg and 1000 mg/kg dose) significantly increased CAT in liver homogenate when compared to CP treated animals. 5-FU treated group also showed significant (P <0.001) increase of CAT when compared to CP treated animals.

Malonaldehyde (MDA): MDA is a measure of lipid peroxidation. MDA level was significantly increase (P <0.001) in case of CP (Control) treated animals when compared with normal control animals. Pretreatment of EETC and aqueous extract of *T. cordifolia* (WETC) (250 mg/kg, 500 mg/kg and 1000 mg/kg body weight) significantly decreased (P<0.05, P<0.01, P <0.001) the MDA level in dose depend manner when it compared with Control. 5-FU treated animals also showed significant (P <0.001) decreases of MDA level when it compares with Control.

Reduced Glutathione (GSH): GSH level was significantly increase in CP treated animals when compared with normal animal. The EETC and WETC (250 mg/kg, 500 mg/kg and 1000 mg/kg) showed significant decrease (P<0.05, P<0.01, P <0.001) in GSH when compared to CP treated animals. 5-FU treated animals also showed significant (P <0.001) decrease in GSH when compared to CP treated animals.

TABLE 3: TABLE SHOWING THE DIFFERENT ENZYME LEVELS

Groups		CAT	MDA	GSH
Normal		7.95±0.25	6.12±0.15	3.56±0.28
Control		4.87±0.32	16.23±0.37	11.2±0.34
5-FU		$7.54±0.45^{***}$	$7.35±0.62^{***}$	$3.81±0.22^{***}$
EETC	250 mg/kg	$5.76±0.27^{ns}$	$14.78±0.26^{ns}$	$10.12±0.48^{ns}$
	500 mg/kg	$6.45±0.41^{*}$	$12.69±0.52^{**}$	$9.5±0.53^{*}$
	1000 mg/kg	$7.1±0.38^{**}$	$11.86±0.43^{***}$	$4.12±0.16^{***}$
WETC	250 mg/kg	$5.95±0.28^{ns}$	$13.1±0.15^{ns}$	$10.50±0.66^{ns}$
	500 mg/kg	$6.8±0.17^{*}$	$11.9±0.48^{**}$	$9.92±0.61^{*}$
	1000 mg/kg	$7.32±0.08^{**}$	$10.6±0.33^{***}$	$4.42±0.78^{***}$

All values are expressed in mean ± SEM (n=6), ns P>0.05, * P<0.05, ** P<0.01, *** P<0.001).

Micronucleus Assay: Ethanolic and aqueous extracts of *TC* in the dose level of 250mg/kg, 500 mg/kg and 1000 mg/kg were studied for anti-mutagenic activity. Cyclophosphamide causes damage to DNA, which leads to formation of micronucleus, shown in **Fig. 4**. An essential parameter for assessing the antimutagenic activity of the Cyclophosphamide-induced model is the number of micronuclei that form. The results obtained for rats treated with different concentrations of *TC* in combination with cyclophosphamide as well as *TC* alone are shown in **Table 4**. No significant difference in the frequency of MNBMC was observed between rats

treated with 1000 mg/kg of *TC* (Group-VI and IX) and the normal control (Group-I). A high increase in the frequency of MNBMC was detected in rats treated with 75 mg/kg of CP (Group-III) compared to the normal control (Group-I) ($p< 0.001$). Simultaneous treatments with different concentrations of *TC* with cyclophosphamide (Group IV-IX) led to reduction in the frequency of MNBMC compared to Cyclophosphamide alone (Group-III), which was significant for the treatments using 250, 500 and 1000 mg/kg of *TC* with the addition of Cyclophosphamide (*p<0.05, **p< 0.01 and ***p< 0.001). These results indicate a dose-response correlation, *TC* was found to be effective and a gradual increase in concentration is proportional increase in the reduction of mutagen city. This result indicates a lack of toxicity to bone marrow.

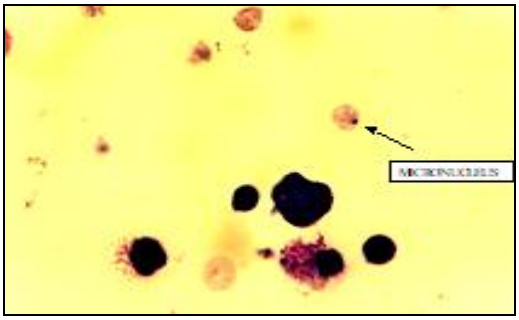

FIG. 4: PHOTOGRAPH SHOWING MICRONUCLEUS (MN) IN BMC

TABLE 4: FREQUENCY OF MNBMC IN RATS TREATED WITH *TINOSPORA CORDIFOLIA* AND/OR CYCLOPHOSPHAMIDE AFTER 30 HR

Groups	MNBMC (Mean ± SEM)
Normal	0
Negative Control	21.66±0.95***
5-FU	2.83±0.30
EETC-250	13.5±0.61**
EETC-500	8.5±0.42***
EETC-500	4.5±0.34***
WETC-250	11.9±0.11**
WETC-500	7.2±0.66***
WETC-1000	4.1±0.39***

Values represent mean ± SEM for each group of 06 rats. Statistical significance *(p < 0.05), ** (p < 0.01), *** (p < 0.001) MNBMC, micronucleated bone marrow cells.

HPTLC Analysis of Extracts: In the present study, chromatographed four different extracts of *TC* by TLC densitometric method using silica gel HPTLC. Solvent system of toluene: ethyl acetate (9:3, v/v) gave well defined peaks of distinct R_f Values.

Chromatogram of Various extracts such as Benzene extract, Ethyl acetate extract, Ethanol extract and aqueous extract and the Rf values obtained are shown in **Fig. 5-8**. The chromatograms obtained can be considered as standard for the identification of *Tinospora cordifolia.*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.00	7.1	0.00	85.6	12.85	0.03	9.6	756.2	2.85
2	0.52	17.7	0.55	27.5	4.13	0.58	24.3	907.7	3.42
3	0.67	33.4	0.71	46.7	7.01	0.72	41.8	1211.4	4.57
4	0.74	45.1	0.79	143.2	21.49	0.80	136.2	3814.1	14.38
5	0.80	136.5	0.89	363.3	54.53	0.94	5.0	19831.4	74.78

FIG. 5: R_F VALUES OF BENZENE EXTRACT

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.00	4.8	0.01	44.3	6.72	0.03	0.9	317.6	1.11
2	0.04	0.2	0.06	18.5	2.81	0.07	1.2	186.5	0.65
3	0.25	7.8	0.26	13.4	2.04	0.27	7.1	150.8	0.53
4	0.31	11.3	0.35	51.5	7.82	0.39	10.8	1225.8	4.29
5	0.47	18.7	0.56	64.2	9.74	0.59	51.4	3641.6	12.73
6	0.61	43.5	0.62	69.8	10.59	0.63	44.3	972.9	3.40
7	0.70	57.6	0.76	94.7	14.37	0.78	90.3	3583.9	12.53
8	0.78	90.7	0.89	302.6	45.92	0.95	2.3	18522.7	64.76

FIG. 6: R_F VALUES OF ETHYL ACETATE EXTRACT

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.01	2.1	0.02	163.0	18.70	0.08	54.4	5451.6	17.50
2	0.13	49.7	0.15	59.5	6.82	0.18	35.8	1728.4	5.55
3	0.23	38.9	0.26	55.2	6.33	0.30	17.5	1613.7	5.18
4	0.34	20.1	0.41	53.5	6.14	0.42	36.1	1970.7	6.33
5	0.42	38.7	0.42	55.2	6.33	0.44	53.5	623.6	2.00
6	0.55	41.0	0.57	43.4	4.98	0.57	18.2	566.7	1.82
7	0.58	23.6	0.58	40.9	4.69	0.59	39.3	374.7	1.20
8	0.64	45.5	0.73	165.1	18.93	0.75	120.5	6132.5	19.68
9	0.78	142.6	0.84	223.3	25.60	0.90	13.6	12551.0	40.29
10	0.91	0.2	0.91	13.0	1.49	0.93	10.7	141.6	0.45

FIG. 7: R_F VALUES OF ETHANOLIC EXTRACT

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	-0.02	0.4	-0.01	164.9	14.64	-0.00	144.6	1615.8	6.40
2	0.00	139.5	0.02	410.4	36.44	0.09	22.9	8923.4	35.34
3	0.37	11.7	0.41	20.0	1.78	0.42	0.0	436.9	1.73
4	0.42	5.1	0.44	24.5	2.17	0.45	22.1	370.2	1.47
5	0.53	25.3	0.57	31.0	2.75	0.58	1.4	719.4	2.85
6	0.58	12.0	0.58	32.5	2.88	0.59	31.3	356.9	1.41
7	0.60	30.9	0.63	37.7	3.35	0.64	21.1	934.0	3.70
8	0.68	46.3	0.74	93.7	8.32	0.76	70.5	3455.9	13.69
9	0.78	72.5	0.82	131.1	11.64	0.83	127.7	3200.7	12.68
10	0.83	116.1	0.84	145.3	12.90	0.90	16.6	4740.7	18.78
11	0.91	2.1	0.92	19.2	1.70	0.95	4.8	342.7	1.36
12	0.95	6.2	0.95	16.0	1.42	0.97	10.9	150.1	0.59

FIG. 8: R_F VALUES OF AQUEOUS EXTRACT

Histopathological Studies: Histopathological studies of rat liver tissue from Group I animals show normal hepatic cells with central vein (V) and sinusoidal dilation **Fig 9A**. In CP treated group (Group II), severe hepatotoxicity was observed by severe necrosis (N) with disappearance of nuclei **Fig. 9B**. The liver taken from Group III animals treated with standard drug 5-FU showed the normal hepatic cells with portal vein (V) and portal artery **Fig. 9C**. Mild degree of necrosis (N) with areas of inflammation adjacent to necrosised area was observed in Group IV animals, treated EETC (250mg/kg/day) **Fig. 9D** while normal hepatocytes with regenerating hepatocytes and mild inflammation in the portal area (M) was observed with Group V animals at the dose of 500mg/kg/day of EETC **Fig. 9E**. At a dose of 1000 mg/kg of EETC, the drug effectively prevented the CP induced liver damage **Fig. 9F**. Similar hepatoprotective effects were seen in the case of WETC in the doses of 250mg/kg, 500 mg/kg, 1000 mg/kg **Fig. 9G, 9H, 9I.**

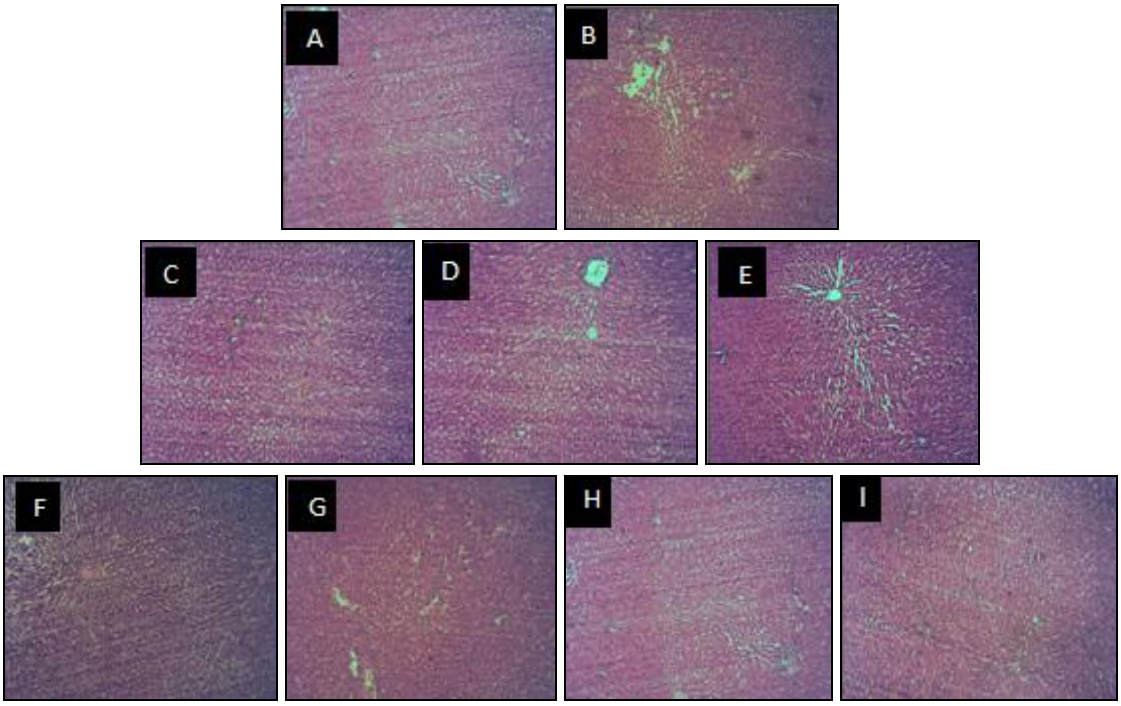

FIG. 9: (A) HISTOPATHOLOGY OF NORMAL GROUP: TREATED WITH SALINE, (B) HISTOPATHOLOGY OF CONTROL GROUP: TREATED WITH CP, (C) HISTOPATHOLOGY OF STANDARD: TREATED WITH CP, (D) HISTOPATHOLOGY OF 250 MG/KG EETC: TREATED WITH CP ALONG WITH *TINOSPORA CORDIFOLIA* LEAVES EXTRACT, (E) HISTOPATHOLOGY OF 500 MG/KG EETC: TREATED WITH CP ALONG WITH *TINOSPORA CORDIFOLIA* LEAVES EXTRACT, (F) HISTOPATHOLOGY OF 1000 MG/KG EETC: TREATED WITH CP ALONG WITH *TINOSPORA CORDIFOLIA* LEAVES EXTRACT, (G) HISTOPATHOLOGY OF 250 MG/KG WETC: TREATED WITH CP ALONG WITH *TINOSPORA CORDIFOLIA* LEAVES EXTRACT, (H) HISTOPATHOLOGY OF 500 MG/KG WETC: TREATED WITH CP ALONG WITH *TINOSPORA CORDIFOLIA* LEAVES EXTRACT, (I) HISTOPATHOLOGY OF 1000 MG/KG WETC: TREATED WITH CP ALONG WITH *TINOSPORA CORDIFOLIA* LEAVES EXTRACT

DISCUSSION: The present studies were aimed to assess and ensure the Anticancer and antioxidant activity of extracts of *T. cordifolia.* In the present study, phytochemical investigation of Benzene, Ethyl acetate, Ethanolic and Aqueous extracts of *T. cordifolia* showed the presence of alkaloids, glycoside, flavonoids, phenolic and tannins, carbohydrates, phytosterols and saponin compounds. The maximum possibility is that the presence of flavonoids, tannins and phenolic compound may show anticancer and antioxidant activity of extracts of *T. cordifolia*. The data obtained in the Total phenolic and Total flavonoid content of the extracts indicate the presence of anticancer and antioxidant activity in the Ethanolic and Aqueous extracts due to higher level of phenolic and flavonoid content. The anticancer agent Cyclophosphamide is a hydrophilic and is sensitive towards the exposure of the light and also prone for oxidation. It is toxic to the liver cells as it can generate toxic ROS during redox cycling in the presence of reducing agents such as Glutathione

and cysteine. Its genotoxic potential for the Bone marrow cells has also been proved. In the present work, involvement of free radicals in the progression of disease and protective effects of *T. Cordifolia* has been examined. The ethanolic and aqueous extracts of *T. cordifolia* have shown the increase in the protective effects with the increase of the dose of the extracts (250mg/kg, 500 mg/kg, 1000mg/kg body weight).

CP is known to increase the oxidative stress in animal models by conversion to its active metabolites aldophosphamide, phosphoramide mustard and highly reactive carbonium ion in liver. These metabolites increase the free radical level and give rise to generation of ROS which further increases the oxidative stress. In Bone marrow cells (BMC's), carbonium ion formed by CP reacts with the extremely electron-rich centres of nucleic acids and proteins. It has undergone thorough testing to produce reactive oxygen species, micronuclei, DNA damage, and dominant lethal mutation (ROS). The most deleterious effect of the free radical generation includes the DNA damage which can further lead to cancer [25, 26].

The radicals are capable of binding to proteins and lipids or abstracting a hydrogen atom from unsaturated lipids, which induces lipid peroxidation and leads to changes in the endoplasmic reticulum, reduction in protein synthesis and elevation of serum transaminase enzymes levels. Increased reactive oxygen species generation is the cause of the LPO increase.In the present study, we observed MDA formation, the index of lipid peroxidation, was significantly increased in liver of CP treated animals. Ethanolic and aqueous extracts of *T. cordifolia* supplementation potentially reduced MDA levels, suggesting that extracts of *T. cordifolia* might have antioxidant principles to produce such response [27]. The present data indicate that i.p. administration of CP disrupts actions of antioxidant enzymes. The decreased activities of these enzymes may be due to the production of reactive oxygen species (ROS) such of superoxide ($O2^{-\bullet}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) that reduces the activity of these enzymes. In the present study, Ethanolic extracts of *T. cordifolia* potentiated the enzymatic antioxidant activities. The enzymatic antioxidant defence system is the nature protector against lipid peroxidation. LPO, CAT and GSH enzymes are important scavengers of superoxide ion and hydrogen peroxide. These enzymes inhibit the production of hydroxyl radicals and guard the components of the cell against oxidative damage.

Catalase (CAT) is a part of endogenous enzymatic antioxidant system, which remains scattered in abundance in animal tissue and highest activity is found in red cells and liver. Hydrogen peroxide is broken down by CAT, which also shields the tissue from extremely corrosive hydroxyl radicals. Similarly, if there is a deficiency of this enzyme, then many harmful effects can occur in the body because due to its deficiency, superoxide radicals and hydrogen peroxide start accumulating [28]. In the present study, Ethanolic and Aqueous extracts of *T. cordifolia* increased the levels of CAT in CP induced liver damage in rats to prevent the accumulation of excessive free radicals and protected the liver from CP intoxication. On the other hand, the extracts reduced the levels of LPO and GSH in CP induced animal models.

In CP induced rats, histopathological studies revealed severe necrosis and the loss of nuclei. This could be due to the formation of highly reactive radicals because of oxidative threat caused by CP. All these changes were very much reduced histopathologically in rats treated with ethanolic and aqueous extracts of *T. cordifolia*. Based on the above results, it could be concluded that ethanolic and aqueous extracts of *T. cordifolia* exerts significant hepatoprotection against CP induced toxicity [29, 30].

CONCLUSION: *T. cordifolia* leaves is claimed to be useful in cancer and oxidative stress. The ethanolic and aqueous extracts of the leaves of *T. cordifolia* showed significant anticancer and antioxidant effect in CP treated rats after oral administration, thus strengthens the claim made by the traditional Indian system of medicine regarding the use of plant leaves in treatment of cancer and oxidative stress. In conclusion, the result of the present study indicates that *T. cordifolia* may have active principle(s) that exerts anticancer and antioxidant activity. However, more efforts are still needed for the isolation, characterization and biological evaluation of the active principle(s) of the *T. cordifolia* extract.

ACKNOWLEDGMENTS: We are thankful to Department of Pharmacy, Guru Ghasidas Vishvavidyalaya (GGV), Bilaspur, (C.G.) for support.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, Znaor A, Soerjomataram I and Bray F: Global cancer observatory: cancer today. Lyon: International Agency for Research on Cancer; 2020. Cancer Tomorrow 2021.
2. Karidio DI and Sanlier SH: Reviewing cancer's biology, An eclectic approach. Journal of the Egyptian National Cancer Institute 2021; 33: 1-7.
3. Luo M, Zhou L, Huang Z, Li B, Nice EC, Xu J and Huang C: Antioxidant Therapy in Cancer, Rationale and Progress. Antioxidants (Basel) 2022; 11(6): 1128.
4. Dehelean CA, Marcovici I, Soica C, Mioc M, Coricovac D, Iurciuc S, Cretu OM and Pinzaru I: Plant-Derived Anticancer Compounds as New Perspectives in Drug Discovery and Alternative Therapy. Molecules 2021; 26: 1109.
5. Yao JN, Zhang XX, Zhang YZ, Li JH, Zhao DY, Gao B, Zhou HN, Gao SL and Zhang LF: Discovery and anticancer evaluation of a formononetin derivative against gastric cancer SGC7901 cells. Investigational New Drugs 2019; 37: 1300-8.
6. Wang WS and Zhao CS: Formononetin exhibits anticancer activity in gastric carcinoma cell and regulating miR-542-5p. Kaohsiung J Med Sci 2021; 37(3): 215-225.
7. Zoi V, Galani V, Lianos GD, Voulgaris S, Kyritsis AP, Alexiou GA: The Role of Curcumin in Cancer Treatment. Biomedicines 2021; 9(9): 1086.
8. Yao Y, Lin M, Liu Z, Liu M, Zhang S and Zhang Y: Hesperidin inhibits lung cancer *in-vitro* and *in-vivo* through pinx1. Front Pharmacol 2022; 13: 918665.
9. Li Y, Domina A, Lim G, Chang T and Zhang T: Evaluation of curcumin, a natural product in turmeric, on Burkitt lymphoma and acute myeloid leukemia cancer stem cell markers. Future Oncology 2018; 14(23): 2353-60.
10. Zhao H, Zhu W and Zhao X: Efficacy of Epigallocatechin-3-Gallate in Preventing Dermatitis in Patients With Breast Cancer Receiving Postoperative Radiotherapy: A Double-Blind, Placebo-Controlled, Phase 2 Randomized Clinical Trial. JAMA Dermatol 2022; 158(7): 779–786.
11. Sferrazza G, Corti M, Brusotti G, Pierimarchi P, Temporini C, Serafino A and Calleri E: Nature-derived compounds modulating Wnt/β-catenin pathway: A preventive and therapeutic opportunity in neoplastic diseases. Acta Pharmaceutica Sinica B 2020; 10(10): 1814-34.
12. Xu L, Yu J, Zhai D, Zhang D, Shen W, Bai L, Cai Z and Yu C: Role of JNK activation and mitochondrial Bax translocation in allicin-induced apoptosis in human ovarian cancer SKOV3 cells. Evidence-Based Complementary and Alternative Medicine 2014.
13. Sharma P, Dwivedee BP, Bisht D, Dash AK and Kumar D: The chemical constituents and diverse pharmacological importance of *Tinospora cordifolia*. Heliyon 2019; 5(9).
14. Chaudhary N, MB and Siddiqui SK: Pharmacognostical evaluation of *Tinospora cordifolia* (Willd) Meirs and identification of biomarkers. J Res Indian Med 2014; 13: 543–50.
15. Khandelwal KR: Practical Pharmacognosy, Techniques and Experiments. 2nd ed. pune: Nirali Prakashan 2000; 149–155.
16. Kokate CK: Practical Pharmacognosy. 5th ed. New Delhi: Vallabh Prakashan 2007; 110–111.
17. Khor BK, Chear NJ, Azizi J and Khaw KY: Chemical composition, antioxidant and cytoprotective potentials of carica papaya leaf extracts: A comparison of supercritical fluid and conventional extraction methods. Molecules 2021; 26(5): 1489.
18. Schlede E: Oral acute toxic class method: OECD Test Guideline 423. Rapporti istisan 2002; 41: 32-6.
19. Medrano-Padial C, Puerto M, Prieto AI, Ayala N, Beaumont P, Rouger C, Krisa S and Pichardo S: *In-vivo* genotoxicity evaluation of a stilbene extract prior to its use as a natural additive: A combination of the micronucleus test and the comet assay. Foods 2021; 10(2): 439.
20. Zahra KF, Lefter R, Ali A, Abdellah EC, Trus C, Ciobica A and Timofte D: The Involvement of the Oxidative Stress Status in Cancer Pathology: A Double View on the Role of the Antioxidants. Oxid Med Cell Longev 2021; 2021: 9965916.
21. Karim MA, Islam MA, Islam MM, Rahman MS, Sultana S, Biswas S, Hosen MJ, Mazumder K, Rahman MM and Hasan MN: Evaluation of antioxidant, anti-hemolytic, cytotoxic effects and anti-bacterial activity of selected mangrove plants (*Bruguiera gymnorrhiza* and *Heritiera littoralis*) in Bangladesh. Clinical Phytoscience 2020; 6(1): 1-2.
22. Souilah N, Ullah Z, Bendif H, Medjroubi K, Hazmoune T, Hamel T, Öztürk M, Nieto G and Akkal S: Phenolic Compounds from An Algerian Endemic Species of *Hypochaeris laevigata* var. hipponensis and Investigation of Antioxidant Activities. Plants (Basel) 2020; 9(4): 514.
23. Hasperué JH, Rodoni LM, Guardianelli LM, Chaves AR and Martínez GA: Use of LED light for Brussels sprouts postharvest conservation. Sci Hortic (Amsterdam) 2016; 213(1974): 281–6.
24. Maataoui SB, Maataoui RB, Almesrar B and Hilali S: Anti ulcer activity of prickly pear (*Opuntia ficus indica*) cladodes extracts. International Journal of Advanced Research 2018; 6(11): 498-506.
25. Ayza MA, Zewdie KA, Yigzaw EF, Ayele SG, Tesfaye BA, Tafere GG and Abrha MG: Potential Protective Effects of Antioxidants against Cyclophosphamide-Induced Nephrotoxicity. Int J Nephrol 2022; 2022: 5096825.
26. Krishna G, Fiedler R and Theiss JC: Simultaneous evaluation of clastogenicity, aneugenicity and toxicity in the mouse micronucleus assay using immunofluorescence. Mutat Res Lett 1992; 282(3): 159–67.
27. Chu H, Yu H, Ren D, Zhu K and Huang H: Plumbagin exerts protective effects in nucleus pulposus cells by attenuating hydrogen peroxide-induced oxidative stress, inflammation and apoptosis through NF-κB and Nrf-2. International Journal of Molecular Medicine 2016; 37(6): 1669-76.
28. Scott MD, Lubin BH and Zuo LKF: Erythrocyte defense against hydrogen peroxide: preeminent importance of catalase. J Lab Clin Med 1991; 1: 7–16.
29. Kamble SS and Gacche RN: Evaluation of anti-breast cancer, anti-angiogenic and antioxidant properties of selected medicinal plants. European Journal of Integrative Medicine 2019; 25: 13-9.

30. Amssayef A and Eddouks M: Antihyperglycemic, antihyperlipidemic and antioxidant effects of cotula cinerea (del) in normal and streptozotocin-induced diabetic rats. Endocr Metab Immune Disord Drug Targets 2020; 20(9): 1504-1513

How to cite this article:
Samal PK, Sharma M, Vaishnaw B, Tiwari A, Sen K, Nirmalkar D and Kumar P: Phytochemical screening, antioxidant and *in-vitro* anticancer activities of *Tinospora cordifolia* leaves extracts. Int J Pharm Sci & Res 2024; 15(5): 1485-96. doi: 10.13040/IJPSR.0975-8232.15(5).1485-96.