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Antioxidant Activity and Anticancer Study on Phytochemicals Extract from Tubers of Gloriosa superba against Human Cancer Cell (Hep-G2)

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ABSTRACT

Gloriosa superba is an alkaloid plant containing a large amount of alkaloid components like colchicine and gloriosine. This study involves anti-cancer examination on Hep-G2 cells (human liver cancer cells) using phytochemical extract obtained from *G. superba* tuber. *G. superba* tubers were identified and collected. The collected sample is shade dried and subjected to pulverisation. The powdered sample is followed for the solvent extraction using soxhlet apparatus for 23 cycles for 48 h in the corresponding temperature of the solvent used. The solvents which are used for the extractions are methanol, water and petroleum ether. Phytochemical qualitative analysis was done. The quantitative analysis was followed to the specific phytochemicals such as saponins and alkaloids which have the anti-cancer properties mentioned from earlier literature studies. The analysed solvents are subjected to anti-oxidant activity by DPPH assay. From each solvent the methanolic extract hold the higher value of anti-oxidant property. The solvent which has the highest anti-oxidant property were preceded to the anti-cancer test against Hep-G2 cells (human liver cancer cells) by MTT assay. The cell death percentage of Hep-G2 cells were calculated from cell viability obtained by MTT assay. Triplicate values were obtained. From the triplicate value the mean value was determined to obtain the average value for each concentration 5 µg, 10 µg, 25 µg, 50 µg, 100 µg. The higher concentration of 100 µg has the lower viable rate and 50% inhibition of viability (IC_{50}) was determined graphically. Death rate of cells indicated the cancer inhibition rate which is due to Hep-G2 cells failed to retain the viability. Inhibition rate of 100 µg has the higher inhibition range of 54.3%. Death rate of Hep-G2 cells may be due to various reasons like protein binding, DNA replication interaction, receptor binding inhibitors, etc. this current study shows the *G. superba* tubers has the potential to inhibit the growth of Hep-G2 cell.

INTRODUCTION

Plants are natural sources of bioactive compounds to treat life threatening diseases such as heart diseases and cancer [1]. *Gloriosa superba* belongs to Liliaceae and is a well-known species of perennial herb. It is also called as “glory lily”. This plant *G. superba* Linn has various phytochemicals, mostly alkaloids like colchicine, gloriosine, colchine, etc. which means that it can be used for treating cancer [2,3]. Seventy-five percentages of the raw materials can be used in this plant; so far the parts of the plants such as tubers, leaves, seeds and flowers are used. These parts of the plants are used in Ayurveda and Yunani as a reputed medicine [4]. *G. superba* plant has various medicinal properties and each part of the plant is used to treat diseases [5]. Plants have anti-oxidant molecules in form of phytochemicals that protect our cells from damage caused by free radicals [6]. *G. superba* contains numerous phytochemicals like alkaloids, glycosides, flavonoids and saponins, which can serve as anti-oxidant and it may reduce the risk of cancer and improve heart health [7]. Cancer is a group of diseases involving abnormal cell growth with the potential of spreading to other parts of the body by invading cells [8]. Hep-G2 is an immortal cell line which is consists of human liver carcinoma cells. They can secret many plasminogen proteins; stimulated by human growth hormone [9]. Cancer is a deadly disease; more than 100 types of cancer affects humans. Treating cancer is so painful process as they involve chemotherapy and radio therapies. Phytochemicals does many works in our body. Based upon their work it can also be anti-cancerous agent

for various types of cancer. Plant source for treating cancer may reduce painful outcomes unlike from chemotherapy [1]. The Phytochemicals from *G. superba* can act as anti-oxidant and anticancer by hormonal action, enzymes stimulators, physical action (contact within the cells) and interference with DNA replication.

METHODOLOGY

Collection of the Plant Material

G. superba tubers were collected from cultivation field around Ariyalur and Perambalur district of Tamil Nadu. About 5 kg of tubers were collected and authenticated by Head of the Department, Meenakshi Ramasamy Arts and Science College, Tamil Nadu, India. Then tubers were pulverized obtaining 1.4 kg of sample powder, the extraction was done using soxhlet apparatus in Greens Med Lab, Chennai.

Preparation of Plant Extract

The air dried plant material was cut into small pieces and dried in shade for two days till it completely dried. Then it was pulverised into fine powder. The 500 g of powdered materials were packed in soxhlet apparatus and successive extraction was performed using petroleum ether, methanol and water solvents. The solution of the extract was filtered through Whattman filter paper no. 1 and concentrated using rotary flash evaporator and dried under vacuum.

Phytochemical Analysis

Phytochemical screening was performed to assess the qualitative chemical composition of different samples of crude extract using commonly employed precipitation and coloration reactions to identify the major secondary metabolites like alkaloids, flavonoids, glycosides, Proteins, phenolic compounds, saponins, starch, steroids, tannins and terpenoids.

The phytochemical analyses were carried out using standard procedures. The extracts of *G. superba* were screened for the presence of secondary metabolites using the procedures. The observations were recorded for total starch, soluble protein, steroids using steroid test, flavonoids and tannins using shinoda test, alkaloids by Dragendroff's test, proteins and glycosides by Limbermann's tests, saponins using forth test, total phenol by ferric chloride test and reducing sugar using Benedict's test.

Quantitative Analysis

Quantification of the alkaloids and saponins can help to understand the test for antioxidant and anti-cancer. Quantitative analysis was done for alkaloids and saponins in an applied standard procedure.

Anti-Scavenging Activity

The free radical scavenging activity of the solvent extracts was determined by 1,1-diphenyl-2-picryl-hydrazil (DPPH). Antioxidant activity was measured. To a fresh tube add 3 ml of methanol which act as a blank. To the second tube add 1 ml of methanol and 3 ml of 0.1 M DPPH. This serves as the control. To the other tubes add respective volume of the sample and make up to 1 ml with Methanol and finally add 3 ml of 0.1 mM DPPH then vortex. Seal the tube with aluminium foil and incubate all the tubes in dark for 30 min at room temperature. Read the absorbance at 517 nm. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%)=[(ABS control–ABS sample)/(ABS control)] × 100

where ABS control is absorbance of negative control and ABS sample is the absorbance of the reaction mixture containing the sample extract.

In vitro Assay for Cytotoxicity Activity (MTT Assay)

The Cytotoxicity of samples on Hep-G2 cells were determined by MTT assay. Cells (1 × 10^5/well) were plated in 1 ml of medium/well in 24-well plates (Costar Corning, Rochester, NY). After 48 h incubation, the cell reaches the confluence. Then, the cells were incubated in the presence of various concentrations of the methanol extract in 0.1% DMSO for 48 h at 37°C. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 200 µl/well (5 mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide cells (MTT) phosphate- buffered saline solution was added. After 4 h of incubation, 0.04 M HCl/isopropanol were added. Viable cells were determined by the absorbance at 570 nm. Measurements were performed and the concentration required for 50% inhibition of viability (IC_{50}) was determined graphically. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks.

The effect of the samples on the proliferation of Hep-G2 was expressed as the % cell viability, using the formula:

% Cell viability=A570 of treated cells/A570 of control cells × 100

RESULT AND DISCUSSION

The main objective of this study is to analyse the potential of tuber extract of *G. superba* to prevent Hep-G2 cancer cells by MTT assay [10]. *G. superba* is an alkaloid plant containing a large amount of alkaloid components like colchicine and gloriosine.

Other than alkaloids various phytochemicals compounds like terpenoids, glycoside, tannin, phenol etc., are also present in lower concentration [4]. These phytochemicals were extracted in solvents such as methanol, water and petroleum ether using soxhlet extraction method [11]. The phytochemicals were analysed for qualitative **(Table 1)** and quantification of alkaloid and saponin. Quantity of alkaloid (1.1311 g/100 g) and saponin (1.7285 g/100 g) were obtained in **Table 2**.

Phytochemical were qualitatively analysed in three different solvents extracts (methanol, petroleum ether, water). Standard procedure was followed and the presence of various phytochemicals was detected by colouration and precipitation. i.e. ('++' presence; '+' faint; '-' negative).

Table 1. Qualitative analysis of phytochemicals.

Phytochemical	Chemical test	Solvents		
		Methanol	Petroleum Ether	Water
Alkaloids	Dragendroff's test	++	+	++
Flavanoids	Shinoda test	+	++	+
Glycosides	Liebermann tests	+	++	++
Saponins	Froth's test	++	-	++
Steroids	Steroids test	++	-	-
Phenols	Ferric chloride test	++	+	-

Alkaloids and saponins were qualitatively analysed with standard procedure to check the quality of the specimens. Quantitative analyse was done directly from plant material.

Table 2. Quantitative analysis for alkaloids and saponins.

Test for Phytochemicals	Quantitative Values (g/100 g)	Test Method
Alkaloids	1.1311 g/100 g	Whattman Filter Paper
Saponins	1.7285 g/100 g	Soxhlet Apparatus

The solvent extracts are followed with DPPH assay to obtain the anti-scavenging of free radicals [12]. The values of water (100 µl: 41.48%) & (200 µl: 65.42%), petroleum ether (100 µl: 46.45%) & (200 µl: 58.95%) and methanol (100 µl: 91.04%) & (200 µl: 91.75%) were obtained **(Table 3)**. Anti-scavenging of different extract was determined from calculating the OD value obtained from absorbance in 517 nm and calculation.

Table 3. Anti-scavenging activity by DPPH assay.

Extract	Test (µl)	Absorbance Value (517 nm)	Percentage of anti-scavenging
Methanol	Test 1 (100 µl)	0.101	91.04%
	Test 2 (200 µl)	0.093	91.75%
Petroleum Ether	Test 1 (100 µl)	0.604	46.45%
	Test 2 (200 µl)	0.463	58.95%
Water	Test 1 (100 µl)	0.66	41.48%
	Test 2 (200 µl)	0.39	65.42%

The methanolic extract has the higher value (100 µl: 91.04%) & (200 µl: 91.75%). As the anti-scavenging is the important factor to prevent cancer and heart disease [5]. MTT test were followed using the extract with higher anti-oxidant value [8]. Hep-G2 cells are used in MTT assay with different concentration of the methanolic extract 5 µg, 10 µg, 25 µg, 50 µg and 100 µg. A triplicate value was obtained using UV-Spectrophotometry analysis of formazan formation [13]. Cell viability percentage was obtained from calculation of absorbent using a formula **(Figure 1)**.

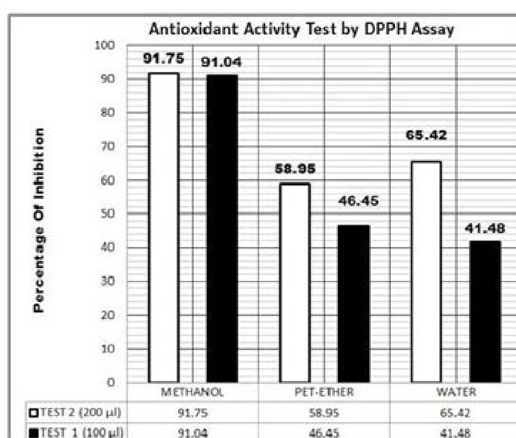

Figure 1. Bar diagram for anti-scavenging activity by DPPH assay.

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DPPH assay was performed to analyse the antiscavenging property of solvent extract from *G. superba* tubers. Each solvent was tested with two concentration 100 µl/ml as test 1, 200 µl/ml as test 2 and bar diagram was plotted from values mentioned in **Table 3**.

Hep-G2 Cytotoxicity Analysis

MTT (3-(4, 5–dimethylthiazol–2–yl)–2, 5-diphenyltetrazolium bromide) assay, it is a homogenous cell viability assay works on the principle of formazan formation. Formazan are measured using UV-spectrophotometer [14]. The viable cells convert MTT into formazan due to its active metabolism, producing colouration which acts as a marker indicator of viability of cells. Formazan crystals are solubilized for absorbance [14].

Hep-G2 is a human liver cancer cell line, used in *in vitro* studies to analysis cytotoxicity of compounds. In this study Hep-G2 cells are used to analyse the cancer inhibition potential of *G. superba* tuber extract. Plant extract with higher antioxidant property are used to analyse the cytotoxicity assay on Hep-G2 cells, antioxidant are good cancer preventers. Methanolic extract of *G. superba* holds the higher value of antioxidant activity hence methanolic extract were used to analyse the MTT assay with different concentration [10].

Concentration of 5, 10, 25, 50, 100 µg/ml methanolic sample was tested for cytotoxicity activity on Hep-G2 cancer cell line **(Figure 2)**.

G. superba tuber's methanolic extract was tested on Hep-G2 cells with different concentration of 5, 10, 25, 50 and 100 µg/ml. The colouration of the cell is due to the formation of formazan crystals. The microscopic view, show the cell death in different concentration. The **Figure 2A**: control, shows Hep-G2 cell line and **Figure 2F**: 100 µg/ml shows the higher inhibition rate of Hep-G2 cells.

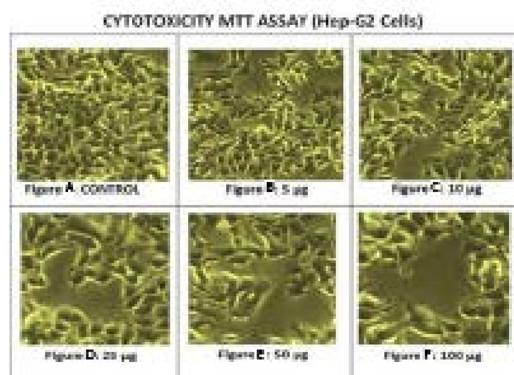

Figure 2. Microscopic view of MTT assay on Hep-G2 cells.

The variant concentration shows triplicate value from absorbance using UV–Spectrophotometry at 570 nm. The absorbance of each concentration shows the viable cell rate with indicating colour of formazan crystals and 5 µg (0.854, 0.856, 0.85), 10 µg (0.739, 0.741, 0.735), 25 µg (0.642, 0.645, 0.638), 50 µg (0.522, 0.524, 0.52), 100 µg (0.436, 0.438, 0.432) were obtained.

The absorbent value was then calculated for total viability of Hep-G2 cells form various concentration. Using standard formula, total cell viability % is obtained in triplicate value **(Figure 3)**.

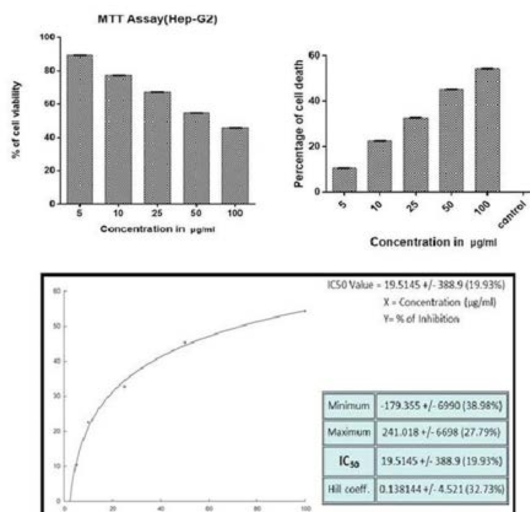

Figure 3. Bar diagram for cell viability & inhibition of Hep-G2 cell by MTT assay and IC_{50} graph for inhibition rate %.

The bar diagram was plotted from the triplicate value of cell viability which mentioned in **Table 4**. Measurements were made and IC_{50} graph was plotted with linear regression curve for 50% of inhibition of Hep-G2 cells, IC_{50}=19 and 5 µg (89.51782, 89.72746, 89.09853), 10 µg (77.46331, 77.67296, 77.04403), 25 µg (67.2956, 67.61006, 66.87631), 50 µg (54.71698, 54.92662, 54.50734), 100 µg (45.70231, 45.91195, 45.28302) were obtained **(Table 4)**.

The variant concentration shows triplicate value from absorbance using UV–Spectrophotometery at 570 nm. Percentage of triplicate viability value was obtained from calculating the standard formula.

Table 4. Cell viability in triplicate value.

Tested Concentrations (µg/ml)	Percentage of cell variability (triplicate value)		
5	89.518	89.727	89.099
10	77.463	77.673	77.044
25	67.296	67.61	66.876
50	54.717	54.9266	54.5073
100	45.7023	45.912	45.283
Control	100	-	-

Cell viability was obtained higher from lower the concentration applied, 5 µg has higher viable cells and the higher concentration of 100 µg has the lower viable rate. Death rate of cells indicated the cancer inhibition rate which is due to Hep-G2 cells failed to retain the viability [15].

Mean values (M ± SD) for the triplicate values of 5 µg (89.4479 ± 0.32022), 10 µg (77.3934 ± 0.32023), 25 µg (67.2606 ± 0.36812), 50 µg (54.7169 ± 0.20964), 100 µg (45.6324 ± 0.32024) measurements were performed and the concentration required for 50% inhibition of viability (IC_{50}=19.5145 ± 388.9) (19.93%) was determined graphically. The inhibition rate was calculated from subtracting the viable cell to total control 100% from each concentration (Mean-control). Inhibition rate of 5 µg (10.5520), 10 µg (22.6065), 25 µg (32.7393), 50 µg (45.2830), 100 µg (54.3675) were obtained **(Table 5)**, where 100 µg has the higher inhibition range of 54.3%. From evaluation of IC_{50} value (19.93%) cell death indicates the cancer inhibition.

Mean average value of viability % from triplicate value; n=3, M ± SD. Inhibition rate % was determined from (mean–control).

Table 5. Average value of cell viability and inhibition rate.

Concentration (µg/ml)	Average value of viability % (M ± SD)	Inhibition rate % (mean-control)
5	89.44972 ± 0.32022	10.552
10	77.39343 ± 0.32023	22.607
25	67.26066 ± 0.36812	32.78
50	54.71698 ± 0.20964	45.283
100	45.63243 ± 0.32024	54.3676

This study concludes the information on *G. superba* tubers has the potential to inhibit Hep-G2 (human liver cancer cells) cell line.

CONCLUSION

The plants are natural source of bio-active compounds which can treat various diseases and life threatening cancer [1]. The *G. superba* methanolic extracts reveals the presence of different types of phyto constituents which has the capacity of anti-oxidant and cytotoxicity effect on Hep-G2 cells. Thus *G. superba* has the potentiality to inhibit the human carcinoma cell line growth.

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CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

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