Evaluation of Anti-Oxidant and Anti-Cancer Activity of Cleome Gynandra Leaves Extract on Hepg2, HT29 And A549 Cells.

SivaKumar Ramalingam[1], Lazarus Vijune Lawrence[2], Renuka Saravanan[3]*
[1, 2, 3*]Department of Chemistry and Biosciences, SASTRA Deemed to Be University, Srinivasa Ramanujan Centre, Kumbakonam 612 001, Tamil Nadu, India.
*Corresponding Author: - Renuka Saravanan
*Department Of Chemistry and Biosciences, SASTRA Deemed to Be University, Srinivasa Ramanujan Centre, Kumbakonam 612 001, Tamil Nadu, India
E-mail: renuka@src.sastra.ac.in
Mobile: 91- 9944256413

Abstract

Background and Objectives: Cancer is a metabolic and signalling disorder that results in unregulated cell division and survival. Despite different treatment strategies, each year the number of cancer cases is increasing every year. Chemotherapy and radiation therapy, in turn cause several side effects. Traditional medicines have demonstrated numerous promising effects in regulating cancer. Among them, Cleome gynandra, is a fascinating plant with high therapeutic potential. Method: This present study focused on investigating the antioxidant activity and cytotoxic effect of leaf extracts of Cleome gynandra against various cell lines such as HT-29, HepG2, and A549. Results: The anti-oxidant status revealed that the chloroform extract of Cleome gynandra possesses good scavenging activity of 82.24%. Based on MTT assay, the selected plants exhibit a potential cytotoxic effect with an IC50 value at 23.64 μg/ml concentration. Conclusion: Cleome gynandra is an excellent medicinal herb and could be used as a potential anti-cancer and anti-oxidant agent.

Keywords: Anti-cancer, Antioxidant, Cell lines, Cleome gynandra, MTT assay.

1. Introduction

The human system is composed of many millions of cells that are ultimately microscopic. These cells are living units of life. Each of these cells has an essential role in everyday life activities. A typical cell undergoes cell division and growth and must die by apoptosis. When these normal cells grow uncontrollably, they become cancer cells. Cancer is a metabolic and signalling disorder that results in unregulated cell division and survival [1]. Cancer is one of the vast and extensive ranges of diseases that can affect any part of a region in the body. One of the specialized aspects of cancer is the formation of aberrant cells very rapidly. This allows the body to grow beyond its normal limits. It can then invade their surrounding and nearby areas of the body and migrate to other parts of organs through metastasis. Breast, lung, prostate, and colorectal cancers are the most predominant types of cancer. In 2020, there were 18,094,716 million cancer cases diagnosed worldwide. According to predictions, there will be around 1,918,030 new instances of cancer in the US in 2022, along with 609,360 cancer-related fatalities. This is due to lung cancer, a primary cause of death that may lead to about 350 deaths daily is due to lung cancer [2].

Several intrinsic or extrinsic factors cause different types of cancer. The most common risk factors for cancer are diabetes, stroke, heart disease, and chronic respiratory diseases. One of the most significant risk factors for causing cancer is tobacco [3]. Tobacco smoking is the most critical contributor to the development of cancer. Approximately 22% of cancer death rates are due to tobacco usage. Several studies also reported that 10% of cancer cases are raised due to obesity and poor diet. It also noted that lack of exercise or excessive alcohol consumption might also be a factor in cancer. Other factors include exposure to environmental contaminants, ionizing radiation, and even certain diseases [4]. These factors are responsible for gene alteration in the DNA of the cells. Cancers caused by inherited genetic defects account for 5–10% of all cases.

The most commonly treated therapies are chemotherapy, hormone therapy, surgery, radiation therapy, immunotherapy, and targeted therapy. These are currently used as cancer treatments. Radiation therapy and chemotherapy were the two most common forms of conventional cancer treatment [5]. Chemotherapeutic drugs, which were cytotoxic to tumors, were the first revolutionary methods in pharmacology for curing cancer. Several unique and novel approaches have been developed in recent years to prevent the spread of cancer. These effective novel therapies are considered better efficient than prior chemotherapeutic drugs [6]. Since various approaches and therapies are available for cancer treatment, cancer is still one of the leading causing diseases. Also, several studies reported many side effects in patients treated with

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chemotherapy and modern drugs.

The discovery of new therapeutic drugs leads to high activity and low toxicity for cancer therapy is aided by traditional medicine. Medicinal plants are significant for their medicinal purposes and for offering biological, economic, and cultural services. Medicinal plants have been the world's primary means of curing ailments. Many plants can fight against several infections. Plants have been a rich source of efficient and safe remedies since ancient times [7]. Many anticancer medicines extracted from several plant components are being investigated on cells (including cancer cell lines) and experimental animals [8]. Some of the principal secondary metabolites include anthraquinones, alkaloids, flavonoids, saponins, steroids, tannins, and terpenoids found mainly in medicinal plants capable of treating various illnesses in the human system. Medicinal plants can produce a different and wide variety of chemical substances preferably used to carry out some essential functions. Many of these phytochemical constituents have many beneficial effects. Some of the components may also show long-term health and could be highly efficient in curing or treating various illnesses of humans [9]. Cleome gynandra L. is a highly notable medicinal and therapeutic plant with numerous applications.

A broad area of research from epidemiological and laboratory studies of Cleome gynandra L. has shown many nutritional aspects. These plant ingredients with highly efficient therapeutic values over the past years. Additionally, this Cleome gynandra is a highly effective plant that has been used as an anti-diabetic anti-inflammatory, antioxidant, anti-cancerous, and immunomodulatory agent [10].

2. Materials and Methods

2.1 Plant collections

The leaves of *Cleome gynandra* were collected from Kumbakonam, Thanjavur District, Tamil Nadu, India. The leaves were shade dried, powdered with an electrical blender, and stored in an airtight container till use.

2.2 Plant Extract Preparation

The freshly dried leaves of Cleome gynandra were washed well with distilled water and shade dried for 15 days. The leaves were ground into powder using a blender and stored in an air-tight container and maintained at room temperature until use. The extraction was done by cold maceration method, 100g of the powdered material was mixed with 200 mL of chloroform with intermittent shaking for 72 h. This extract was then filtered and evaporated for one hour in a water bath at 60°C.

2.3 Antioxidant Activity [(2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay]

The antioxidant activity of Cleome gynandra leaves extract was determined DPPH free radical scavenging method performed by Anitha et al. 2020 [11]. Different concentrations of (50-100 µg/mL) of leaves extracts were made up of distilled for 1mL. About 2mL of 0.1 mM methanolic solution of DPPH was mixed. Three ml of DPPH solution served as control and ascorbic acid was used as standard. The reaction mixture was incubated and maintained at room temperature for 30 mins. After the incubation, the colour intensity was measured at 517 nm in a spectrophotometer. The experimental procedure was performed in triplicates. A decrease in the absorbance of DPPH solution indicates an increase in free radical scavenging activity. The results were calculated and compared with the positive control (Ascorbic acid). The following formula was used to determine the extract's radical scavenging efficiency.

$$\text{DPPH scavenging activity}(\%) = \left[\frac{(Abs_{control} - Abs_{sample})}{Abs_{control}}\right] \times 100$$

Abs of sample is the absorbance of DPPH free radical + test sample, whereas Abs of control is the absorbance of DPPH free radical + chloroform. IC50 values were used to present the findings.

2.4 Cell culture

2.4.1 HT-29 Cell culture

Adenocarcinoma cells isolated from the human colon are known as HT-29. The HT-29 cells were purchased from National Centre for Cell Sciences (NCCS), Pune, India. Using 96 well plates, the cells were plated with a concentration of 1×104 cells/well in Dulbecco's Modified Essential Medium (DMEM media). The media was supplemented with 1X AAS and 10% FBS. The HT-29 cells were cultured and maintained in the CO2 incubator at 37˚C with a humidified atmosphere of 5% CO2 and 95% air. All reagents required for the cell culture were purchased from Himedia, Mumbai, India.

2.4.2 HepG2 Cell culture

HepG2 is a human hepatocellular liver cell line (perpetual cell line). The cell line (HepG2) was obtained from the NCCS, Pune, India. The cells were plated separately using 96 well plates with 1×104 cells/well in DMEM media. The media was supplemented with 1X AAS and 10% FBS. The HepG2 cells were cultured and maintained in the CO_2 incubator at 37˚C with a humidified atmosphere of 5% CO2 plus 95% air.

2.4.3 A549 Cell culture

The A549 cells, primary lung tumor cells, were obtained from the NCCS, Pune, India. The cells were plated separately using 96 well plates. It is plated with 1×10^4 cells/well concentration in DMEM media. The media was supplemented with 1X AAS and 10% FBS. The A549 cells were cultured and maintained in the CO_2 incubator at 37˚C with a humidified atmosphere of 5% CO_2 plus 95% air. All reagents required for the cell culture were purchased from Himedia, Mumbai, India.

Cell Growth Inhibition Study – MTT Assay

In order to assess the anti-proliferative activity and inhibitory concentration (IC50) of plant extracts on

various cell lines HT-29, HepG2, and A549), the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] test was performed [11]. The cells were grown in a 96-well plate using MEM (1X104 cells/well) for 48 hours at 85% confluence. A new medium containing a substance that had been serially diluted was used to replace the old one. The cells were then incubated for 48 hours. After removing the culture media from each well, 100 µL of the MTT solution was added. The mixture should be incubated for 4 hours at 37 °C. The formazan crystals were solubilized by adding 50µL of DMSO isopropanol to each well after removing the supernatant and incubating for 10 minutes. In an ELISA multi-well plate reader, the absorbance was measured at 620 nm (Thermo Multiskan EX. USA). The percentage of viability was determined using the reported absorbance and the following formula:

$$\% \text{ of cell viability} = \frac{OD\ Value\ of\ test}{OD\ value\ of\ control} x\ 100$$

3. Results and Discussion

In various countries, traditional medicine uses medicinal plants for treating many diseases and ailments. An essential and fundamental stage in determining the effectiveness of plant extracts on a clinical level is extensive scale screening of crude extracts. Several findings indicate that the plant crude extracts show proliferation at tumor locations when there is a lack of oxygen, triggering the host's immune system and gene-expressing regulation to inhibit tumor growth [12]. Approximately 25% of modern medications are obtained directly from plants or sometimes indirectly, demonstrating the strong foundation for plant-based derived medicine. Even in India, modern systems of medicine are available, and a considerable section of the population finds the promising effects of various alternative medications including Ayurvedic or Unani systems [13]. C. gynandra is one of the most commonly utilized medicinal plants used to treat cancer. Hence, for the present investigation, this plant was selected and evaluated for its antioxidant and anti-cancer cytotoxic potential by in vitro studies.

Free radicals are molecular species that are formed in living organisms. These radicles are also found externally and are known to induce a variety of degenerative illnesses such as mutagenesis, carcinogenesis, cardiovascular problems, and aging. The substances used to reduce or fight against free radicals are called antioxidants. These substances fight against free radicals by interfering with the free radical-mediated oxidative process. The DPPH free radical scavenging assay is the first method for determining the antioxidant capacity of a chemical in extracts or other biological sources [14].

Figure 1 represents the results of the DPPH radical scavenging activity of leaf extracts of C. gynandra. The extract was found to possess the free radical scavenging ability with the IC50 value of 82.24%. Prior studies reported that chloroform extract of C. gynandra possessed lysosomal protective effects, antioxidant activity, and anti-inflammatory properties in adjuvant-induced arthritic rats. The ability of a plant extract to scavenge DPPH radicals determines the antioxidant activity of the selected extracts [15]. Free radical production has been linked to the development of several disorders including cancer. For instance, phytonutrients that can scavenge free radicals have incredible promise in defending against diseases, including cancer, rheumatoid arthritis, diabetes, and other illnesses. The results obtained in the free radical scavenging activity of leaf extracts in C. gynandra were closely similar to Partha 2018. Moyo et al. (2018). Their studies concluded that C. gynandra has higher antioxidant activity as compared with Beta vulgaris and Brassica oleracea [16].

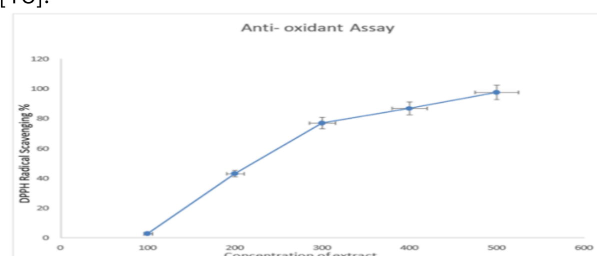

Figure 1. DPPH free radical scavenging activity of chloroform extract of

Cleome gynandra leaves Cell viability has been evaluated using the MTT assay frequently. However, it is essential to remember that mitochondrial succinate dehydrogenase catalyzes the enzymatic conversion (reduction) of 3-[4,5-dimethyltiazole-2-yl]-2-5diphenyltetrazolium bromide (MTT) to MTT-formazan. The MTT assay relies on mitochondrial respiration and analyses a cell's capacity to produce energy indirectly. Cell monolayers plated in 35 mm multiwall plates can readily be used as a colorimetric reaction for the MTT assay [17]. This present study aims to evaluate the efficacy and activity of Cleome gynandra leaves extracts against three different cell lines such as HT29, HepG2, and A549.

HT29 is the human colon adenocarcinoma cell line. From human colon cancers, numerous cell lines have been produced. These cell lines were initially used to evaluate several facets of human cancer biology. HT29 cells have frequently been employed to investigate the possible anticancer effects of different dietary supplements and their gastrointestinal digests based on the HT29 cell line's carcinoma genesis [18]. Other activities, such as immunomodulatory, antioxidant, and barrier-protecting characteristics, have been broadly investigated in HT29 cells. This may also be researched in the derived cell lines of HT29.

Moreover, HT29 cells are a well-established model for studying the intestinal epithelial response against many other bacterial infections. HT29 cell line exhibits enterocyte characteristics and can be used for attachment. These cells secrete mucin, the mucus layer has been implicated in influencing the adherence of live organisms along with bacterial

components such as lipopolysaccharides to the epithelial surface [19].

Figure 2 represents the cytotoxic effect of chloroform extract of Cleome gynandra leaves against HT29 cell line. The cell growth inhibitory activity (IC50) of leaves extract in the plant was found to be at 23.64 µg/ml

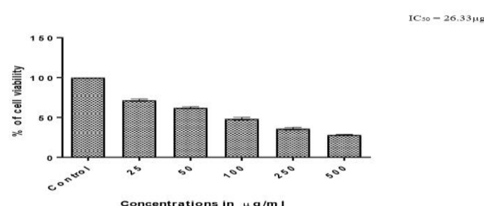

Figure 2. Cytotoxic effect of chloroform extracts of Cleome gynandra on HT29 cell line

The liver performs detoxification in the human body and is primarily responsible for drug metabolism and drug interactions; hepatic cells play an essential role in drug development and discovery. Owing to this, HepG2 cells are commonly utilized in drug development and toxicity testing [20]. HepG2 cell line is a widely used cell line for a hepatic tumor isolated from hepatocellular carcinoma. Several studies used this cell line as it has a wide range of applications in research [21]. Various therapeutic approaches, such as chemotherapy, radiation therapy, and surgery, are presently performed to treat liver cancer. Plant sources, on the other hand, can give innovative and effectual anti-liver cancer phytochemicals and evaluate their antioxidant activity [22].

Figure 3 depicts the cytotoxic effect of chloroform extracts Cleome gynandra. leaves against the HepG2 cell line. The IC50 of the leaves extract was found to be at 46.08 µg/ml.

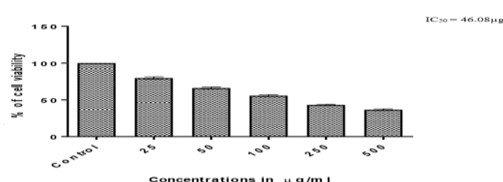

Figure 3. Cytotoxic effect of chloroform extract of Cleome gynandra leaves on HepG2 cell line

In lung cell biology, the human A549 adenocarcinoma cell lines have been considered a representation of Alveolar epithelial type II (ATII) cells. The highly specialized ATII cells create surfactant, a versatile lubricant. This lubricant lowers the surface tension. Also, it avoids alveolar collapse at the time of ventilation. This cell line was developed in 1972 by Giard *et al.* for type 2 pneumocyte lung cancer [23]. Surfactant generation and surfactant system regulation have been studied in vitro using A549 cells.

Figure 4 represents the graphical representation of the anti-cancer cytotoxic effect of chloroform extracts in the leaves of Cleome gynandra. L., against the HepG2 cell line. The cell growth inhibition study results [inhibitory activity (IC50)] of leaves extract in the plant was noted at a concentration of 26.33 µg. Chloroform extract displayed the lowest (IC50) value among these three cell lines in the A549 cell line against the other two cell lines. The results showed that 23.64 µg of the Cleome gynandra chloroform extract significantly inhibited the growth of the A459 cell line.

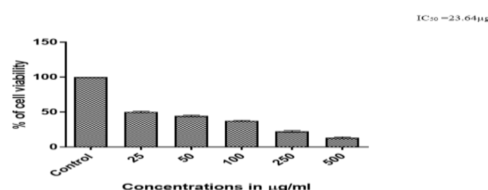

Figure 4. Cytotoxic effect of chloroform extract of Cleome gynandra leaves on A549 cell line

In-vitro studies of chloroform extract in the poly-herbal formulation were conducted to establish its cytotoxic impact on the Vero cell line. Trypan blue dye exclusion could also be used to evaluate the % of viable cells. The anti-cancer cytotoxicity assay was performed using an MTT assay [24]. Besides, similar prior studies have demonstrated that these phytoconstituents can help in preventing metastasis by inhibiting cancer cell growth [25]. From this present evaluation, it was concluded that the chloroform extract of Cleome gynandra leaves show significant antioxidant and anti-proliferative activities. The bioactive compounds present in the leaves extract could have attributed for the cytotoxic effect against the cancer cells.

4. Conclusion

The antioxidant activity and anti-cancer cytotoxic effect of chloroform extract of *Cleome gynandra* leaves against HT29, Hep2, and A549 cell carcinoma were investigated. Based on the results, this study confirmed that chloroform extract of *Cleome gynandra* leaves on A549 cell line have potential cytotoxic effect compared to HT-29 and HepG2 cell line. From the data obtained, it may be concluded that the *Cleome gynandra* leaves could be used as an alternative source for the management of cancer.

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