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IN-VITRO ANTIOXIDANT AND ANTI-PROLIFERATIVE EFFECTS OF ETHANOLIC LEAF EXTRACTS OF COSTUS COSMOSUS LINN ON HepG2 CELL LINES

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Abstract

Medicinal herbs have long been used to treat a variety of ailments. India's traditional medicinal system relies heavily on the use of medicinal herbs. Plants have natural compounds that have antiproliferative properties against a variety of malignancies. Cancer is a disease that kills people and causes major defects in their bodies. There are numerous different types of cancer disorders that affect different organs in humans. There is no effective treatment for these types of cancers. In the present study, ethanolic extracts of *Costus cosmosus* have been investigated for its *in-vitro* antioxidant and cytotoxic potential against HepG2 cell lines. The ethanolic extracts of *Costus cosmosus* exhibited significant cytotoxic action against cell lines, as measured by cellular death in a dose-dependent manner, with the strongest effect occurring at higher doses. The findings suggested that the anticancer properties of ethanolic leaf extracts of *Costus cosmosus* could be linked to their flavonoid concentration. This research validates the cytotoxic potential of *Costus cosmosus* against HepG2 cell lines.

Keywords: *Costus cosmosus,* HepG2 cell lines, antioxidant, *In-vitro* cytotoxic potential.

Introduction

Hepatocellular carcinoma (HCC) is one of the most frequent cancers on the planet, with an estimated prevalence of one in every 100 persons. As seen by the increasing mortality rate, it is becoming an increasingly prominent cause of death. The American College of Cardiology has said that The chance of getting HCC is higher in patients who have extensive fibrosis, usually cirrhosis, and hepatitis B infection. Individuals with chronic hepatitis B and C infections are the ones who are most prone to acquire the condition. Treatment options, such as liver resection, liver transplantation, systemic and local therapy, must be tailored to the individual needs of each patient to be effective. The understanding of the probable connections between different etiological factors that contribute to the development of HCC, as well as the current diagnosis and treatment approach, is necessary to improve the screening, early detection, prevention, and management of hepatocellular carcinoma.

Plants have been utilized as cures for thousands of years, and botanical literature has detailed the use of plant extracts in many applications. A variety of medicinal plants are utilized in the treatment and prevention of cancer in many different countries (Madhuri et al.,2009). Over the years, researchers have concentrated their attention on the anticancer properties of plants (Saluja M.,2011; Muthuraman et al., 2008; Sowemimo et al., 2007). Cragg et al. (2005) discovered that medicinal plants are a good source of anticancer medicines such as Taxol, vincristine, and camptothecin, which are effective. The introduction of new medications has not prevented cancer from becoming the leading cause of death in the world, claiming the lives of nearly 6 million people each year despite these advances (Abdullaev et al., 2000). As a result, there is a strong need to discover novel natural compounds that are more powerful against cancer while also having fewer side effects has become desirable, and natural goods are constantly being researched throughout the world.

Costus comosus Linn is a perennial plant that belongs to the family of costaceae. It is a rhizome that is usually referred to as red tower ginger. It is a popular plant in tropical gardens all over the world because it provides a brilliant display of colour. It is often found in tropical and subtropical regions of Asia, Africa, and the Americas, as well as other parts of the world. Traditionally, the leaves and rhizomes have been used to treat fever, rash, asthma, bronchitis, and intestinal worms, diabetes, and liver diseases (7). Despite these studies and the widespread use of this plant in traditional medicine, the literature review of this plant afford no reported pharmacological works. Hence, the present study aims to evaluate the *in-vitro* antioxidant and cytotoxic potential of leaves of ethanolic leaf extracts of *Costus comosus* (EECC) against HepG2 cell lines.

Methods

Plant Material

The leaves of the plant, *Costus comosus* was collected from the Shervaroy hills, Salem, and from the tribal medical shops in October 2020. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu. The herbarium specimen of the plant (KVCC-1) was maintained in the college museum. The plant parts were shade dried at room temperature for 10 days and coarsely powdered and passed through sieve No.60.

Preparation of Extracts

About 500 g of dried leaves were coarsely powdered and subjected to continuous hot percolation with different solvents of increasing order of polarity such as pet ether, chloroform, acetone, ethanol, and aqueous [8,9]. The extracts were dried under the rotary evaporator and then tested for various phytochemical constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates.

DPPH radical scavenging activity

EECC was tested for their antioxidant activity by the DPPH method (10). The extract (20, 40, 60, 80, 100 µg/mL) was mixed with 3 mL of methanolic solution containing DPPH radicals (0.1 mM). After 30 min, absorbance was determined at 517 nm. The percent inhibition of activity was calculated by using the formula

$$\% \, inhibition = \frac{A_o - A_e}{A_o} \, x \, 100$$

Where, Ao = absorbance without extract; Ae = absorbance with extract.

The results were expressed as IC_{50} which is the concentration of the sample required to inhibit 50 % of DPPH concentration.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay is a novel method to assess the antioxidant power of the sample. The method performed in this study was as described by Prasad *et al*(11). It is based on the ability of antioxidants to reduce Fe^{+3} to Fe^{+2} in the presence of TPTZ (2, 4, 6-tripyridyl-s-triazine), forming an intense blue Fe^{+2} – TPTZ complex. FRAP solution (3 mL) mixed with 100 ml of the EECC and incubated at 37 °C for 10 min. Absorbance was measured at 593 nm for different concentrations (0.2, 0.4, 0.8 or 1 mg/mL) of extract in FRAP reagent. The absorbance of the samples was compared to a $FeSO_4$ standard curve and the FRAP values were expressed as mmol Fe (II)/mg extract

MTT assay

Formazan-based viable cell mass assay (MTT assay)

MTT assay is based on the cleavage of the soluble yellow tetrazolium salt MTT into a blue-colored formazan by the mitochondrial enzyme succinate dehydrogenase. This assay is extensively used for measuring cell survival and proliferation. There is a direct proportionality between the formazan produced and the number of viable cells. However, it depends on the cell type, cellular metabolism, and incubation time with MTT. This method is based on the capacity of mitochondrial enzymes of viable cells to reduce the yellow soluble salt MTT to purple-blue insoluble formazan precipitate which is quantified spectrophotometrically at 570 nm after dissolving in DMSO. Cells are plated onto 96 well plates and allowed to grow in CO2 incubator for 24 hrs (37°C, 5% CO2). The medium is then removed and replaced by a fresh medium containing different concentrations of leaf extract for 48 hrs. The cells are incubated for 24-48 hrs (37°C, 5% CO2). Then, 20 µL MTT stock solution (5 mg/mL in PBS) is added to each well and incubated for 4 hrs. The medium is removed and 200 µL DMSO is added to each well to dissolve the MTT metabolic product. Then the plate is shaken at 150 rpm for 5 minutes, and the optical density is measured at 570 nm [12].

% Cell viability = ([O.D of control–O.D of test compound]/[O.D. of control]) × 100

O.D: Optical density

The results were presented in Table.1

Results

Percentage yield and Phytochemical screening

The leaves of the *Costus cosmosus* Linn were collected from the foothill of Yercaud, Salem in October 2020. The leaves were shade dried and extracted with varying solvents of increasing order of polarity by a continuous hot extraction process using soxhlet apparatus. The average percentage yield of pet ether, chloroform, acetone, ethanol and aqueous extracts of Costus cosmosus was found to be 2.8, 1.4, 1.52, 5.16, and 3.89% w/w respectively. The phytochemical evaluation showed the presence of flavonoids, phenolic compounds, tannins, glycosides, saponins, and carbohydrates in the acetone, ethanol, and aqueous extracts of the plant. Alkaloids and terpenoids were present in chloroform extract. Gums and fixed oils were present in petroleum ether extract. Thus the phytochemical analysis confirmed the presence of bioactive compounds and this may serve as a potential source for the cytotoxic activity. Hence, based on the percentage yield and phytochemical results, ethanolic extract of leaves of Costus cosmosus was selected for its antioxidant and cytotoxic activity.

DPPH radical scavenging activity

The DPPH free radical scavenging activity of the EECC was carried out. The extracts were tested at concentrations of 20, 40, 60, 80, 100 µg/ml. The EECC has shown 94.56% inhibition of the DPPH radical at 100 µg/ml concentration, whereas the standard (Ascorbic acid) has shown 98.42% inhibition at the same concentration. The extract showed the DPPH radical scavenging activity even at the lowest concentration of 20 µg/ml. The DPPH radical inhibition was increasing and concentration-dependent as that of ascorbic acid as the standard compound. The IC_{50} values of the EECC and ascorbic acid were found to be 73.94 µg/mL and 91.24 µg/mL respectively was shown in table.1.

Total antioxidant activity by FRAP method

In- vitro total antioxidant activity for EECC was estimated by ferric reducing antioxidant power method using ascorbic acid as standard. The EECC showed the Frap activity of 810.52±21.42 µmol Fe (II)/g extract, whereas ascorbic acid showed 1045.67±27.12 /µmol Fe (II)/g shown in table.1.

MTT ASSAY

The percentage of survived cells was calculated by measuring the absorbance of respective incubated cells in the 96 wells plate. The effect of EECC on HepG-2 cell lines is significant and comparable to the standard drug Silymarin. The extract has shown the activity cell inhibit in a lower concentration of 12.5 µg/ml whereas cell inhibits in a higher concentration of 200µg/ml of EECC & Silymarin showed 64.25 & 88.6%. Thus the extracts had shown dose-dependent and significant cytotoxic activity. The results were shown in Table.2.

Discussion

Cancer is a group of more than 100 distinct diseases defined by uncontrolled cellular proliferation, local tissue invasion, and distant metastases9, and free radicals have been linked in carcinogenesis10. Many plant extracts containing antioxidant properties have been shown to have antitumor action, which gives support to this claim11. As a result, plants containing flavonoids, glycosides, and other phytochemicals are continually being tested for antitumor action.

The present study investigated the *in-vitro* antioxidant and cytotoxic effect of ethanolic leaf extracts of *Costus cosmosus* against HepG2 cell lines. *Costus cosmosus* (Linn) is a traditional medicinal plant whose medicinal properties were not validated so far. The results of the phytochemical analysis showed the presence of sterols, carbohydrates, saponins, tannins, proteins, terpenoids, phenolic compounds, and flavonoids. It was reported that free radicals are one key factor for carcinogenesis. In this study, the in-vitro antioxidant potential of EECC was evaluated by DPPH and FRAP assay methods and it showed promising antioxidant activity in a dose-dependent manner. In FRAP assay, the ability to reduce Fe^{3+} to Fe^{2+} was also significantly higher at concentrations 0.2–1 mg/mL. It was reported that the phenolic compounds were directly related to the antioxidant property (13).

MTT is a colorimetric assay that measures the reduction of yellow MTT by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilized with an organic solvent (eg. isopropanol) and the released, solubilized formazan reagent is measured spectrophotometrically. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells. In this study, EECC showed promising cytotoxic activity in a dose-dependent manner.

The results of the present study showed the promising in-vitro antioxidant and cytotoxic potential of ethanolic extract of leaves of *Costus cosmosus* on DPPH, FRAP methods, and MTT assay against the HepG2 cell lines in a dose-dependent manner. This may be due to the antioxidant properties of the flavonoids, tannins, and polyphenols present in it. In the future, isolation and characterization studies are required for further evidence of its anticancer activity.

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S.no	Sample	*In-vitro* antioxidant activity	
		DPPH Method (IC_{50} in µgm)	FRAP Assay (µmol Fe (II)/g).
1	EECC	91.24 ±8.24	810.52±21.42
2	Ascorbic acid	73.94±5.68	1045.67±27.12

Table.1. Effect of Ethanolic Leaf Extracts of *Costus Cosmosus* Linn on DPPH and FRAP methods.

S.no	**Concentration µg/ml**	**% Cell inhibition**	
		EECC	**Silymarin**
1	12.5	4.50	11.7
2	25	25.80	35.0
3	50	32.20	68.5
4	100	38.15	80.6
5	200	50.25	88.5

Table.2. Effect of Ethanolic Leaf Extracts of *Costus Cosmosus* Linn on Hepg2 Cell Lines by MTT Assay