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Research Article

The ethanol extract of *Syzygium cumini* exhibits cytotoxic potentials against breast cancer cells due to antioxidant properties

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Abstract

The present investigation was designed to evaluate in vitro cytotoxic potentials of ethanol extract of *Syzygium cumini* against breast cancer cells and also estimate its antioxidant activity. The ethanol extract of *Syzygium cumini* was prepared by successive solvent extraction process using sox-let apparatus. The ethanol extract was evaluated for its in-vitro anticancer potentials against MCF-7 human breast cancer cell lines by MTT assay and concentration of the ethanol extract required to inhibit 50% of cell growth (IC_{50}) was recorded. To determine antioxidant properties of the extract, superoxide scavenging, lipid peroxidation and DPPH methods were used and IC_{50} values of extracts were noted. In current study, ethanol extract of *Syzygium cumini* has shown anticancer property by exhibiting significant IC_{50} values against growth of breast cancer cell growth. The extract has also shown significant IC_{50} values in superoxide scavenging, lipid peroxidation and DPPH methods and antioxidant activity was comparable to standard drug ascorbic acid. The results obtained from the present research work suggested that the ethanol extract of *Syzygium cumini* possess significant *in vitro* cytotoxic potentials against breast cancer cells. The results also suggested that the ethanol extract have *in vitro* antioxidant properties.

Keywords: Anticancer activity, *Syzygium cumini,* IC_{50} value, MTT assay and antioxidant activity.

INTRODUCTION

Cancer is serious and common disease condition which kills peoples in the society more than infectious diseases such as tuberculosis, malaria and HIV/AIDS combined[1]. Cancer has become major health problem worldwide and claims more than 10 million people dies a year due to breast, lung, liver and colon ovarian cancers[2]. According to estimation about 12.7 million cancer cases and 7.6 million cancer deaths are reported in 2008 among which 56% of the cases and 64%of the deaths occurred in the economically developing world[3]. On the Indian scene, about 1.1 million new cancer cases reported per year which stands India as a single country among 184 countries and contributes about 7.8% of deaths to global cancer mortality figures[4]. Cancer has the second highest mortality rate after cardiovascular diseases throughout the world. Even though remarkable progress has been made by medical science, the availability of safe and specific anticancer drugs has remained a major challenge in clinical practice[5]. Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately cancer is projected as the primary cause of death in the future. Antioxidants are a group of substances that are useful for fighting cancer and other processes that potentially lead to diseases such as atherosclerosis, Alzheimer, Parkinson, diabetes and heart disease. Antioxidants act by preventing the onset of cancer during carcinogenesis and they are generally beneficial to cells[6].

Medicinal plants have been used since ancient time in Ayurveda and other traditional medicine system and their utility has been increasing day by day throughout world. Natural compounds obtained from herbs are considerably safe and effective than synthetic compounds. Moreover, the problem of development of drug less compared to synthetic drugs. is also reduced. The herbal drugs comprise a major source of anticancer medicine due to presence of various phyto-constituents such as vincristine, vinblastine, taxanes, camptothecines and in developed countries and developing countries. The free radicals are the main agents which induces mutation by damaging cellular DNA ultimately leads to development of cancers in the body. Hence much attention has been given on the development of anticancer agents that possess antioxidant property due to their free radical scavenging potential which plays an important role in protection of DNA free radical mediated damage[7].

The *Syzygium* is a genus of plant, pantropical taxa with about 400 species distributed chiefly in Asia, Africa, Australia and America[8]. The genus is well known for its richness in prenylated flavonoids and is considered to possess insect repellant, larvicidal, piscicidal, antimicrobial and anticancer

properties. The *Syzygium cumini* belongs to the genus was essentially used for the management of diabetes, cancer, hyperlipidemia, hepatotoxicity and renal problems in the folklore medicine but doesn't have the scientific evidence for the same[9-13]. In this attempt, the study had been conducted to determine *in vitro* antioxidant and cytotoxic potentials of ethanol extract of *Syzygium cumini*

MATERIALS AND METHODS

Preparation of the ethanol extract & phytochemical investigation

The authenticated plant leaves were dried under shade, then powdered and 200gm of powdered drug was defatted with petroleum ether. A part defatted powdered drug was subjected to ethanol extraction in soxhlet apparatus for 48 hours . The preliminary phytochemical investigation for the ethanol extract of (*Syzygium cumini* (SCEE) was conducted[14].

Preliminary phytochemical investigation

The preliminary phytochemical investigation for the ethanolic extract of *Syzygium cumini* had been conducted as per procedure prescribed by Khandelwal[15].

Drugs and chemicals

All reagents and chemicals used in the study were obtained commercially and were of analytical grade. The standard drugs tamoxifen and paclitaxel were obtained as gift samples from Strides laboratories, Bangalore.

Evaluation of antioxidant property

The evaluation of antioxidant activity of ethanol extract of *Syzygium cumini* was carried out by the following two methods:

Lipid peroxidation method

Lipid peroxidation inhibition was estimated by the formation of colored product in the reaction mixture. The assay mixture contained the ethanol extract in various concentrations, to which were added 0.1 ml of potassium chloride (30 mM), 0.1 ml of ascorbic acid (0.06 mm) and 0.1 ml of ammonium ferrous sulphate (0.16 mM) in succession. Later the reaction mixture was treated with 0.2 ml of sodium dodecyl sulphate (8.1%), 1.5 ml of thiobarbituric acid (0.8%) and 1.5 ml of 20 % acetic acid (pH 3.5) and then 5 ml of 15:1 v/v butanol-pyridine mixture was added. The absorbance of the organic layer containing the thiobarbituric acid reactive substances (TBARS) was measured at 532 nm[16].

DPPH radical scavenging activity

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH. About 0.1 mM solution of DPPH in ethanol was prepared and 1 ml of this solution was added to 3 ml of the different concentration of ethanolic extract (50-400 µg/ml) in different test tubes. The mixture was shaken and allowed to stand at room temperature for 30 min and the absorbance was measured at 517 nm using a spectrophotometer. Decrease in absorbance of the reaction mixture indicates higher free radical scavenging activity[16,17].

2.5.3 Superoxide scavenging activity

This method carried out by using Nitro blue tetrazolium (NBT) reagent, the method is based on generation of super oxide radical (O2-) by auto oxidation of hydroxylamine hydrochloride in presence of NBT, during the reaction the NBT is reduced to nitrite. In brief, aliquots of 0.1 to 1.0 mL to ascorbic acid solution were taken in a test tube, to which 1 mL of sodium carbonate, 0.4 mL of NBT and 0.2 mL of EDTA were added and zero-minute reading was taken at 560 nm. The reaction was initiated by the addition of 0.4 mL of hydroxylamine hydrochloride to the above solution. Reaction mixture was incubated at 25^{0}C for 5 mins; the reduction of NBT was measured at 560 nm. A parallel control was also treated in the similar manner. The ethanol extract was treated in the similar manner, absorbance was recorded and IC_{50} values was calculated[16,17].

2.6 Evaluation of anticancer activity

2.6.1 Procurement of cell lines

The MCF-7, human breast adenocarcinoma cell line first isolated in 1970 from the breast tissue of 69 year old Caucasian women used for the present investigation. The MCF-7 cells were obtained from NCCS, Pune and subcultured under suitable conditions[18].

2.6.2 MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assay

The cytotoxicity of EESC against MCF-7, HCT-116, HEP-G2, A-549 and vero cells was determined by the MTT (3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyltetrazolium Bromide) assay Exponentially growing cells were harvested from 25mL flask and a stock cell suspension was prepared. Cells (1 ヲ 105/well) were plated in 100オl of medium well in 96-well plates (Costar Corning, Rochester, NY). After 48 hours incubation the cell reaches the confluence. Then cells were incubated in the presence of various concentrations of the EESC and standard drugs in 0. 1% DMSO for 48 h at 37 ¬C. The tamoxifen was used reference drugs for the breast cancer cell lines and paclitaxel was used standard drugs for other cell lines. After removal of the sample solution and washing with phosphate-buffered saline (pH7 .4), 20オl/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 4h incubation, 0.04M HCl isopropanol was added. Viable cells were determined by the absorbance at 450 nm. Measurements were performed and the concentration required for a 50% inhibition of viability was determined graphically. The absorbance at 450 nm was measured with a UV- Spectrophotometer using wells without sample containing cells as blanks. The effect of the samples on the proliferation of cells was expressed as the % cell viability, using the following formula: % cell viability = A450 of treated cells / A450 of control cells ヲ 100%. The percentage growth inhibition was calculated using the following formula[18].

$$\% \text{ growth inhibition} = \left\{100 - \left(\frac{\text{mean absorbance of individual test group}}{\text{Mean absorbance of control group}}\right) \text{x } 100\right\}$$

The IC50 value obtained is the concentration of sample required to inhibit the growth of 50% of viable cell population.

3.0 RESULTS

3.1 Preliminary phytochemical investigation

The percentage yield of the EESC was found to be 7.91 % w/w. The preliminary phyto-chemical investigation for the ethanol extract of *Syzygium cumini* reveals the presence of poly phenols, flavonoids, tannins, steroids, alkaloids and carbohydrates.

3.2 Determination of *in-vitro* antioxidant activity

In the evaluation of antioxidant activity, ethanol extract of *Syzygium cumini* has shown significant antioxidant property by exhibiting significant IC_{50} values against all the three *in vitro* models such as DPPH method, Lipid peroxidation method

and Superoxide scavenging activity. The IC_{50} values of EESC were comparable to that of ascorbic acid [See Table 1].

Table 1: Effect of ethanol extract of *Syzygium cumini* EESC on, DPPH, Lipid peroxidation and Superoxide scavenging methods

Sample (µg/ml)	I.C50 (µg/ml)		
	DPPH method	Lipid Peroxidation	Superoxide Scavenging
Ascorbic acid	6.72 ± 2.92	8.02 ± 1.2	37.51±0.23
EESC	30.18 ± 2.17	22.67 ± 2.61	51.22±1.15

All the values are expressed as Mean± SEM, n = 6.

3.3 Evaluation of *in-vitro* anticancer activity by MTT assay

In the present study, we used MCF-7 human breast cancer cells for the evaluation of *in-vitro* cytotoxicity potentials The ethanol extract was evaluated by MTT assay against all cell lines. The concentration of extract that required toreduce 50% of absorbance (IC_{50}) was recorded against each cancer cells.

3.3.1 MTT assay against MCF-7 cells

The significant growth inhibition was observed due to presence of standard drug Tamoxifen and EESC of breast cancer cell lines in a study performed to determine the anticancer property of EESC against breast cancer cell lines, the ethanol extract of *Syzygium cumini* has shown percentage of inhibition at 500µg/ml was 73.14 and . Both Tamoxifen and EESC a have exhibited the significant IC_{50} values [Table 2, Figure 1 and Figure 2].

Table 2: The IC50 values of Tamoxifen and EESC against MCF-7 breast cancer cell growth

Treatment	IC_{50} Values
	MCF-7
Tamoxifen	25.802
EESC	194.5

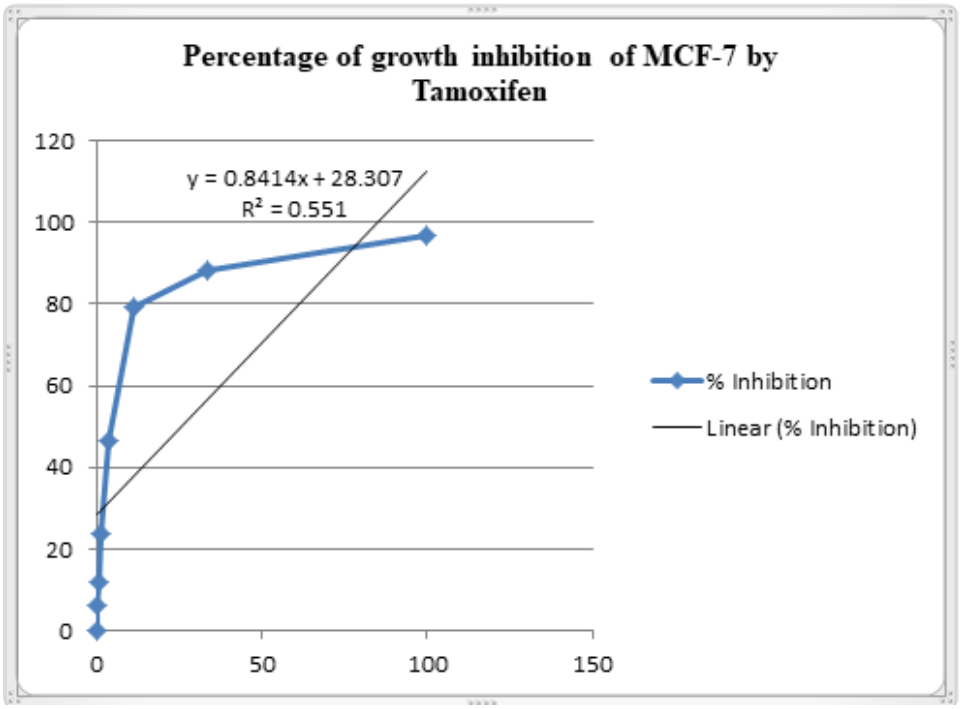

Figure 1: The percentage inhibition of growth of MCF-7 by Tamoxifen

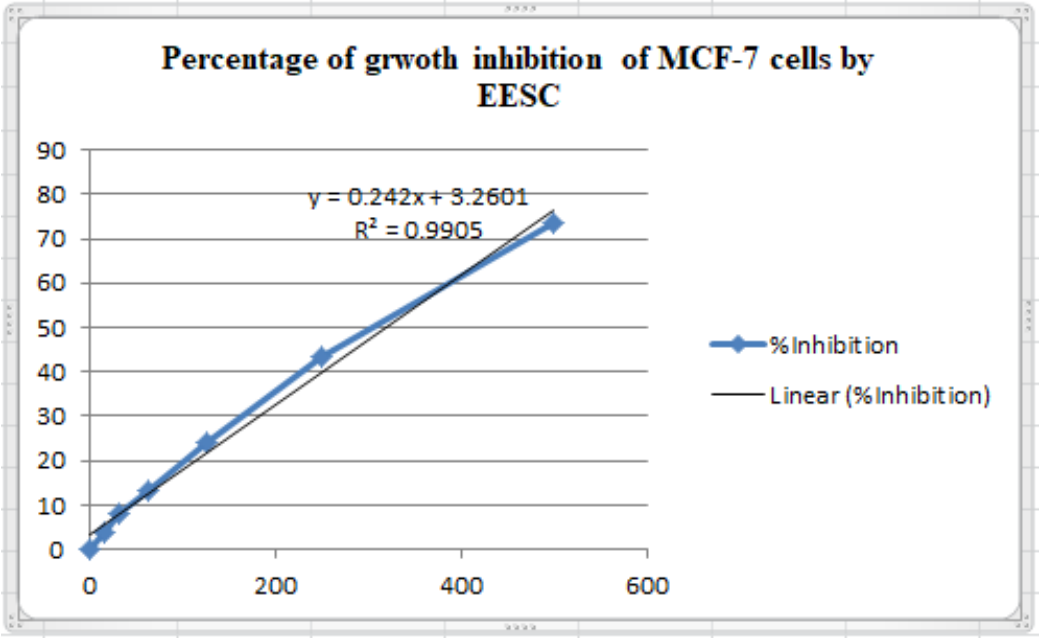

Figure 2: Percentage of grwoth inhibition of MCF-7 cells by EESC

DISCUSSION

Natural products have been regarded as important sources that could produce potential chemotherapeutic agents. Plant derived compounds in particular have gained importance in anticancer therapy and some of the new chemotherapeutic agents currently available for use include paclitaxel, vincristine, podophyllotoxin and camptothecin, a natural product precursor from water soluble derivatives[19,20]. Several epidemiological surveys have shown that a diet rich in vegetables and fruits might give protection against tumors by mechanisms that have not been well established yet but probably due to their antioxidant activity. In recent years, naturally occurring plant substances have been getting increased scope for the intervention of malignant invasive progression in the late stage of cancer diseases. On the basis of this knowledge, certain foods including vegetables, fruits and grains, as well as phyto-constituents of diversified pharmacological properties have been shown to provide a significant protection against various cancers[21,22]. Furthermore, there is an increased scope to establish scientific basis on use herbal agents for the management cancers and humans around the globe probably discovered natural remedies against disease and cancer by trial and error over the millennia. Although there are some new approaches to drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development. Medicinal plants have long been used to prevent and treat many diseases, including cancer due to their antioxidant potentials and thus they are good candidates for the development of anti-cancer drugs[23,24]. In this regard the study was performed to evaluate the anticancer potentials of the ethanol extract of *Syzygium cumini* against human normal, breast, colon, liver and lung cancer cell lines.

MTT [3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide] is taken up by the viable cells and reduced to formazan by enzyme succinate-tetrazolium reductase system that belongs to the mitochondrial respiration chain functioning in metabolically active cells. Formazan formed, is a purple coloured water-insoluble product that is largely impermeable to cell membranes and hence resulting in its accumulation within the healthy cells. Hence intensity of purple color and absorbance depends on dead cells. In the present study, the ethanol extract of *Syzygium cumini* was subjected to preliminary phytochemical investigation and also evaluated for *in vitro* antioxidant and *in vitro* cytotoxic property.

Human breast cancer cells are estrogen receptor (ER)-dependent and carries the wild type tumour suppressor p53 gene. The Tamoxifen is an estrogen receptor antagonist used to treat the breast cancer was used in this study as a reference standard drug. The study revealed that the ethanol extract prepared from the *Syzygium cumini* obtained was effective in attenuating the viable tumor cell count in dose dependent manner and shown significant IC50 value which was comparable to results of standard tamoxifen. According to rule if the crude extract is showing IC_{50} value less than 1mg/mL (1000µg/mL) against MTT assay, then it can be concluding that the plant extract possesses significant cytotoxic property[25,26]. In the present study, ethanol extract has shown IC_{50} values against all the cancer cell lines below 500µg/mL and hence considered as significant values though they are many times more than synthetic standard drugs Tamoxifen and Cyclophosphamide[27]. The *Syzygium cumini* was essential component of Ayurveda a traditional medicinal system of medicine due to presence of various phyto-constituents for the treatment of various health complications such as diabetes mellitus, ulcers, liver diseases, urinary disorders and cancers. In the present study the ethanol extract was proven for its effective anti-oxidant potentials which is the important mechanism required for the anticancer activity[28,29]. The ethanol extract also shows the significant cytotoxicity against various cancer cells while normal cells were not affected by the extract. But further detailed study is necessary to correlate the anti-oxidant and cytotoxic effects of the extract.

Conflict of interest

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

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