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Research Article

In-Vitro Anti Breast Cancer Activity of *Syzygium Cumini* Against MCF-7 Cell Line

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Abstract

In this study, different concentrations of the methanolic extract of fruit pulp of the plant *Syzygium cumini* was subjected to in-vitro cytotoxic activity study against MCF-7cells using the MTT assay. Percentage cell viability of cell lines were carried out by using Trypan blue dye exclusion technique MTT assay was used to evaluate the reduction of viability of cell cultures in the presence and absence of the extract. Cell viability was inhibited to different extents by different concentrations of the extract.

Key words: *Syzygium cumini*; cytotoxic activity; crude extracts; MTT.

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1. Introduction

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases [1, 2]. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics [3,4].

Several commonly used herbs have been identified by the National Cancer Institute as possessing cancer-preventive properties. Those include members of the Allium sp. [garlic, onions and chives]; members of the Labiatae family [basil, mints, oregano, rosemary, sage, and thyme]; members of the Zingiberaceae family [turmeric and ginger]; members of the Umbelliferae family (anise, caraway, celery, chervil, cilantro, coriander, cumin, dill, fennel, and parsley) [5]. In addition, many herbs contain a variety of phytosterols, triterpenes, flavonoids, saponins, and carotenoids, which have been shown from studies of legumes, fruit, and vegetables to be cancer chemoprotective [6].

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Breast cancer is the most commonly occurring cancer in women, comprising almost one third of all malignancies in females. It is second only to lung cancer as a cause of cancer mortality and it is the leading cause of death for American women between the ages of 40 and 55 [7]. The lifetime risk of a woman developing invasive breast cancer is 12.6 % 2 one ut of 8 females in the United States will develop breast cancer at some point in her life [8].

Syzygium cumini (Family-Myrtaceae) is native to India and East Indies. It is commonly called as Jamu Koli in Odia; Black Plum, Java Plum in English and Jamun in Hindi. The plant possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations [9]. Most of these compounds have been reported to possess antioxidant and free radical scavenging activities [10].

2. Materials and Methods

Collection of plant material and extraction

Ripe fruits of *Syzygium cumini* was collected from the forest part of Bhubaneswar hill area situated in the eastern part of India in the month of May and identified by Dr. S K Sahu, a taxonomist at Utkal University, VaniVihar, Odisha, and Dr. S P Panda, Herbarium keeper, Regional Plant Research Center, Bhubaneswar. Voucher specimens were deposited in the herbarium of the Department of Botany, Utkal University. The fruits were cut into small pieces and the pulp was separated from the seeds. The pulp was shade-dried, and milled. The coarsely powdered, shade dried fruit pulp of both the plants was first defatted with Petroleum ether using soxhlet apparatus. The extracts were concentrated using rotary evaporator to get solid residue. The marc from the central compartment was removed, dried and extracted by exhaustive extraction with a series of solvents of increasing polarity with Soxhlet extractor was done [11]. The weight of the residue extracts were recorded and percent yield calculated. Solvents used with increasing polarity are petroleum ether, ethyl acetate, methanol and the scheme of extraction was depicted as a flow chart (Figure 1) below.

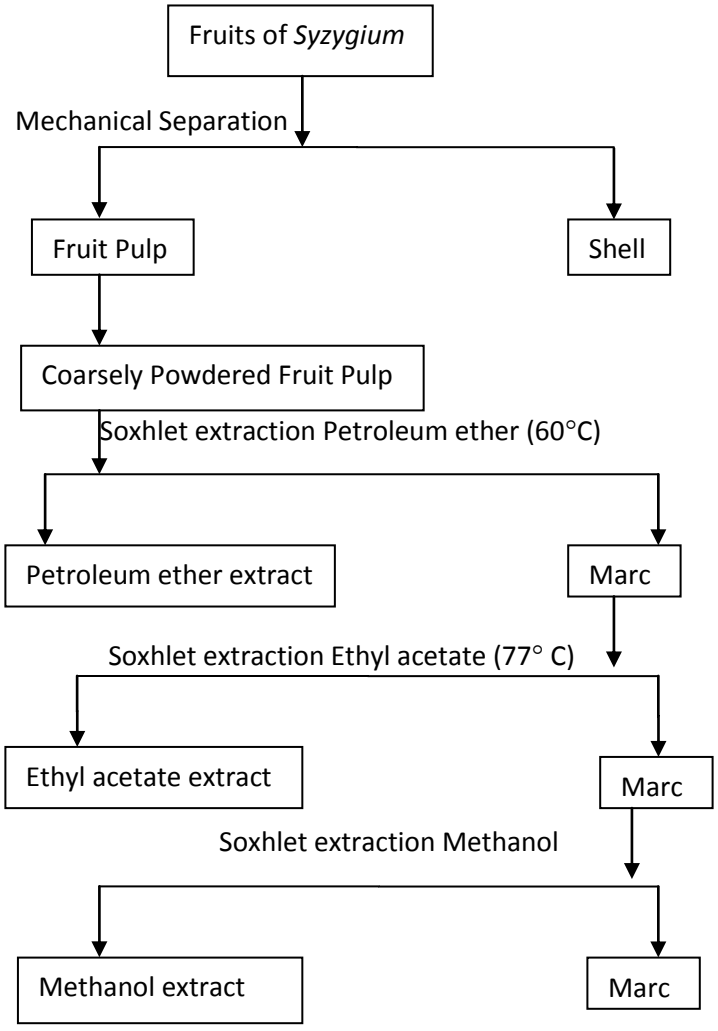


Figure 1: Scheme of extraction for the Fruit pulp of *Syzygium cumini.*

Preliminary Phytochemical Screening

The percentage yield of other extracts except methanolic extract was negligible. So, the methanolic extract was taken for further experimental work. The prepared methanolic extract was subjected to routine phytochemical analysis [12] to identify the presence of various phytochemicals such as carbohydrates, alkaloids, glycosides, saponins, flavonoids, tannins, sterols, phenols, etc.

Cell Culture

Human breast cancer MCF-7 cell line was obtained from Sigma-Aldrich, Bangalore. MCF-7 is a cell line used in many studies due to its characteristics and being easy to culture. The MCF-7 cell line is adherent and grows in clumps. The cells were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

***In Vitro* Anti-Breast Cancer Activity**

Viability Staining by Trypan blue dye exclusion method

Principle

Trypan Blue is a vital blue acid dye that has two azo chromophores group. The reactivity of Trypan blue was based on the fact that the chromophore was negatively charged and does not interact with the cell unless the membrane was damaged. Therefore, all the cells which exclude the dye are viable.

Trypan blue will not enter into the cell wall of plant cells grown in culture. It is used in estimating the number of viable cells present in a population.

Procedure

The Methanolic extract of *Syzygium cumini* (MESC 200 mg/kg) was studied for short term *in vitro* cytotoxicity using MCF-7cells. From the above solutions 10mg of the extracts were taken in an Eppeandorf vial of capacity 1ml and diluted to six different concentrations with its duplicate and control (50%) using DMSO as a solvent and mixed with the help of a vortexing machine. The cell viability was checked by trypan blue dye (4%) [13,14]. The cell suspension ($2x10^6$ cells in 0.1ml) was added to tubes containing various concentrations of the test compounds and the standard drug Doxorubicin. The volume was made up to 1ml using Phosphate Buffered Saline (PBS).

Control tube contained only cell suspension. These assay mixtures were incubated for 3 hour at 37^0C. After incubation 0.1 ml trypan blue was added and number of dead cells determined by using an Automated cell counter. The mixtures were examined for the viability of the cells (non – viable cells are stained and viable cells excluded the stain). Cells were counted by the following formulae:

Cell count = Number of cells ×Dilution (Area ×Thickness of fluid film)

Percentage of cell viability = (Live cell count/Total cell count) ×100

Micro culture tetrazolium (MTT) assay

Cell viability was assessed by MTT assay (Micro culture tetrazolium/ formazan assay) in the presence and absence of different concentrations of the plants extract. The cells were seeded in 96-well plates. Four wells for each concentration were seeded and triplicate plates were used the cell line. Then, the cells were incubated at 37°C. After 36 h of incubation, various concentrations of fruit extracts were added to the wells to obtain final concentrations of 62.2, 125, 250 and 1000 µg/ml, respectively. Control groups were mixed with DMSO to obtain a final

concentration of 1 %. Doxorubicin was used as positive control. The cells were incubated for an additional 48 h, 50 µl of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] (1 mg/ml, Sigma) in phosphate buffered saline (PBS) was added to each well, and incubated for 4 h at 37 °C. The medium was removed and formazan was dissolved in DMSO and the optical density measured at 590 mm using a bioassay reader (Biorad, USA) [15].

IC_{50} was defined as the concentration of the extract that produced a 50% decrease in cell viability relative to the negative control which was wells exposed to the solvent without any extract [16, 17].

Statistical Analysis

Data are reported as the mean ±SD for at least three replicates. Statistical analysis was performed using the Student t-test, with significance level set at $P < 0.05$.

3. Results and Discussion

The various concentration of plant extract used were 62.5, 125, 250, 500 and 1000 µg/ml, Doxorubicin and control (without extract). Decrease in cell count was observed with increase in concentration of the extracts. There was a dose dependent increase in cytotoxic activity for all the concentrations tested. *In vitro* exposures of MCF-7 cells with various concentrations of *Syzygium cumini* extract (62.5, 125, 250, 500, 1000 µg/ml) significantly suppressed MCF-7 cancer cell growth in a dose-dependent manner ($P<0.05$). The maximum inhibition of MCF-7 cells due to exposure to MESC was found at 1000 µg/ml of the extracts was 70.12% inhibition. The results showed dose dependent response against MCF-7 cell line. The cytotoxic activity may be due to the presence of secondary metabolites like flavonoids present in *Syzygium cumini*.

The result of the present study reveals that, the *in vitro* anti breast cancer activity was observed in Trypan blue exclusion assay and MTT assay against MCF-7 cell line. Although the anti-breast cancer activity of Doxorubicin was more than that of the extracts, it may be noted that the drug is a single entity whereas the extract is a crude one containing numerous compounds.

Treatment and Conc. (mcg/ml)	**Absorbance**	**% Inhibition**	**IC_{50}**	**R^2**
MESC (62.5)	0.427	14.25	266.8	0.9617
MESC (125)	0.380	23.69		
MESC (250)	0.268	46.05		
MESC (500)	0.165	66.73		
MESC (1000)	0.092	70.12		
Standard	0.00021	99.91		
Control	0.528			

Table 1: Determination of Cytotoxicity of MESC

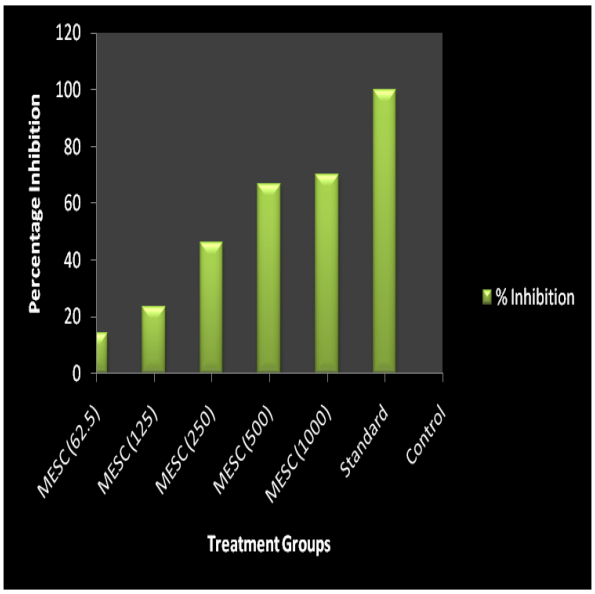


Figure 2 : Percentage growth inhibition of MESC, Control and Standard against MCF-7 cell line.

4. Conclusion

Studies have shown differential sensitivities to several natural compounds between tumor and normal cells *in vitro* or *in vivo*, and the results obtained from the present study show that the methanol extract from fruit pulp of *Syzygium cumini* had anti breast cancer activity against MCF-7 cell lines. Our phytochemical screening revealed the presence of flavonoids, alkaloids, steroids in the methanolic extract of *Syzygium cumini*, which could be responsible for this activity. Flavonoids have been found to possess antimutagenic and antimalignant effects [18, 19]. Moreover it has protective effect against breast cancer by their effect on signal transduction in cell proliferation and angiogenesis [20]. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent against cancer and we therefore, suggest further, the purification and characterization of the phytochemicals along with investigations are needed to provide some additional insight into the *in vivo* cytotoxic activity of the plants with a view to obtaining useful chemotherapeutic agent.

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