



Anti-cancer and anti-oxidative potential of *Syzygium cumini* against breast cancer cell lines

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ABSTRACT

This study was aimed to investigate the phytochemical constituents anti-cancer and anti-oxidative potential of *Syzygium cumini* against breast cancer cell lines. Phytochemical screening of ethyl acetate and methanol extracts of *Syzygium cumini* seeds were analysed by standard method. The antimicrobial activities of ethyl acetate extract of *Syzygium cumini* seeds were investigated. The ethyl acetate, methanol and ethanolic extracts of *Syzygium cumini* seeds investigated against MCF-7 breast cancer cells and antioxidant activity. Phytochemical screening of ethyl acetate and methanol extracts of *Syzygium cumini* seeds have shown the presence of Alkaloids, Steroids, Flavonoids, Saponins, Quinones, tannins and Proteins. The ethyl acetate extract of *Syzygium cumini* seeds has shown moderate activity against 3 pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* and 2 fungi's such as *Candida albicans* and *Aspergillus niger*. The ethyl acetate, methanol and ethanolic extracts of *Syzygium cumini* seeds showed significant antioxidant activity which helps in scavenging the free radicals against MCF-7 breast cancer cells. Ethyl acetate extract of *Syzygiumcumini* has shown the maximum scavenging activity when compared to other two extracts. The results of our study showed that the phyto constituents present in the *Syzygium cumini* seeds could provide potential bioactive compounds for the development of new leads to cancer diseases. Preliminary qualitative phytochemical analysis

Keywords: *Syzygium cumini*, Phytochemicals, MCF-7 breast cancer cells, Antioxidant

INTRODUCTION

Cancer of the breast existed in ancient times and reference to this disease can be found dating back as 3000 BC, in an Egyptian papyrus [1]. Breast cancer refers to cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. It is a clonal disease; a single transformed cell- the end results a series of somatic (acquired) or germ line mutations able to express full malignant potential. Thus breast cancer may exist for a long period as either a non-invasive disease or an invasive but nonmetastatic disease.

Most patients with breast cancer have surgery to remove the cancer from the breast. The main goal of surgical therapy is to remove the cancer and accurately define the stage of disease. Surgical options broadly consist of breast conservation therapy followed by radiation therapy. Breast conserving surgery, an operation to remove the cancer but not the breast itself it includes lumpectomy, partial mastectomy, total mastectomy, modified radical mastectomy, radical mastectomy, radiation therapy, chemotherapy [2]. Even though there are number of synthetic antitumour agents available, efforts are still on to search for effective naturally occurring anticarcinogens that would prevent, slow or reverse cancer development. Plants have a special place in the treatment of cancer. It is estimated that plant derived compounds constitute more than 50% of anticancer agents [3, 4]. Recent studies on tumor inhibitory compounds of plant origin have yielded an impressive array of novel structures. Epidemiological studies suggest that consumption of diets containing fruits and vegetables, which are major sources of phytochemicals and

micronutrients, may reduce the risk of developing cancer. Certain products from plants are known to induce apoptosis in neoplastic cells but not in normal cells [5-8].

Syzygium cumini is rich in compounds containing anthocyanins, glucoside, ellagic acid, isoquercetin, kaemferol and myrecetin. The seeds are claimed to contain alkaloid, jambosine, and glycoside jambolin or antimellin, which halts the diastatic conversion of starch into sugar and seed extract has lowered blood pressure by 34.6%. The greatest amount of anthocyanins and flavonoids leads to more powerful free radical scavenging effect and there by inhibiting the cancer promoting activity as shown by methanolic extract of *Syzygium cumini*. Scientific studies on *Syzygiumcumini* have shown that the various extracts of seed possess a range of pharmacological properties such as antibacterial, antifungal, antiviral, anti inflammatory, anti-ulcerogenic, cardioprotective, anti-allergic, anticancer, radioprotective, antioxidant, hepatoprotective, antidiarrheal, hypoglycemic and antidiabetic effects. The vast number of literatures found revealed that the extracts of different parts of *Syzygium cumini* showed significant pharmacological actions [9]. The present study has been undertaken to explore the possible anti-cancer and anti-oxidative potential of *Syzygium cumini* against breast cancer cell lines.

EXPERIMENTAL SECTION

Collection of plant materials

The seeds of *Syzygium cumini* were collected from local area near Vadapalani Chennai.

Drying

The collected seeds were allowed for shade drying for 1 week completely. The dried seeds were ground into powder, which was used for further extraction.

Preparation of plant extract

Soxhlet extraction

25gms of *Syzygiumcumini* powder was taken and extracted with 250ml of methanol and ethyl acetate using soxhlet apparatus for 2 hours. The extraction was allowed to run for 15 cycles. The extracts were collected in a sterile beaker and allowed drying. The crude extract was scrapped from the beaker and stored in sample vials. The crude extract stored was used for phytochemical, antioxidant, antimicrobial, anticancer and DNA fragmentation studies in cancer cell lines. Qualitative analysis of phytoconstituents [10].

Anti bacterial assay

Agar disc diffusion method

Antibacterial activity of extracts was determined by disc diffusion method on Muller Hinton Agar [MHA]. Medium is poured in to the petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial (*Staphylococcus aureus* *Pseudomonas aeruginosa* and *Escherichia coli*) suspension. The discs were placed in MHC plates and add 20 μ l/disc of sample [Concentration 1000 μ l, 750 μ l, 500 μ l] was placed in the disc. The plates were incubated at 37°C for 24 hrs. Then the antibacterial activity was determined by measuring the diameter of zone of inhibition.

Antifungal assay

Agar disc diffusion method

Antifungal activity of extracts was determined by disc diffusion method on Sabouraud Dextrose Agar [SDA] medium was poured into the Petri plate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal (*Candida albicans* and *Aspergillus niger*) suspension. The disc were placed in SDA plates and add 20 μ l of sample (Concentration:1000 μ l, 750 μ l, 500 μ l) were placed in the disc. The plates were incubated at 37°C for 24hours. Then the antifungal activity was determined by measuring the diameter of zone of inhibition.

DPPH free radical scavenging: (Non-enzymatic assay)

The ability of the extract to scavenge DPPH radical was determined by the method described by [11].

MTT ASSAY [12]

Cytotoxicity of ethanol extracts fresh and *Syzygiumcumini* powder were examined in MCF-7(breast) cell lines.

Cell viability assay: MTT salt was used to determine the viability of the cell lines throughout the study. MTT was dissolved in PBS with concentration of 5 mg/ml directly before use. 100 μ l medium containing 20 μ l solutions was added to each well and the micro plate was incubated at 37°C, 5% C for 4 hours. At the end of incubation period the

medium was discarded gently and replaced by 100 µl of DMSO. The plate was shaken on the microplate shaker to dissolve formazan. Absorbance was recorded at 570 nm.

Media preparation for animal tissue culture:

The Reagents required for media preparation are Sodium Bicarbonate, DMSO, EDTA, Fungi zone, FCS, L – Glutamine, HBSS, Herpes Buffer, Kanamycin, MEM, Penicillin, Streptomycin, Trypsin, and TPVG. On the day of preparation, all the ingredients were brought to room temperature. The pH meter and weighing balance were switched on 10 minutes before starting the media and reagent preparation. The MEM was dissolved in the pre-sterilized Millipore double distilled water, mixed well, closed and sterilized at 121°C, for 15 minutes. It was then cooled to room temperature. The ingredients were added in quantities as indicated in table 1. Depending on the concentration of Foetal Calf Serum (2%, 5% and 10%). The ingredients were mixed well by shaking. The bottle was shaken and the pH was adjusted to the range of 7.2 to 7.4. It was then stored at 4°C. One aliquot of the prepared MEM was kept for 2 days at 37°C and checked for sterility, pH drop and floating particles. They were then transferred to the refrigerator.

Table 1: Volume of Ingredients for Media Preparation

Ingredients	10% Growth Media	5% Maintenance Media	2% Wash Media
MEM	862ml	912ml	942ml
Penicillin (a) & Streptomycin (b) (100 IU of a 100g of b)	1 ml	1ml	1 ml
Kanamycin (20mcg/ml)	1 ml	1 ml	1 ml
Fungi zone (20mcg/ml)	1 ml	1 ml	1 ml
3% L-Glutamine	10 ml	10 ml	10 ml
Foetal Calf Serum (FCS)	100 ml	50 ml	20 ml
75% NaHCO ₃	20 ml	20 ml	20 ml
Herpes buffer (IM)	5 ml	5 ml	5 ml
Total volume	1000 ml	1000 ml	1000 ml

Maintenance of cell lines

The cell line used is MCF-7 (breast cancer) cell line obtained from King Institute of Preventive Medicine, Guindy, and Chennai. The tissue culture bottles that showed confluent monolayer were selected by observing them under an inverted microscope. Growth medium was removed from the bottle, washed with PBS/MEM without FCS and 5 ml of TPVG (for 25 cm²) was added dispersing evenly on the monolayer and left in contact with the cells for 2-3 minutes until there is a cloudy appearance on the monolayer. TPVG was removed and the bottle was incubated at 37° C until all the cells detach from the surface. The cells were resuspended in 5 ml of growth medium (MEM containing 10% FCS). The suspension was aspirated few times to break the cell clumps. The cell suspension was then transferred to a 96 well plate. 100µl of the cell suspension was added to each well. The 96 well plate was then incubated in a desiccator maintained with 5% CO₂ for 72 hours.

Sub culturing of MCF – 7 cell lines

The medium and TPVG were brought to room temperature. The tissue culture bottles were observed for growth, cell degeneration, pH & turbidity. The bottles were selected for splitting. The mouth of the bottle was wiped with cotton soaked in spirit. The medium was removed using 10ml pipette. The cells were gently rinsed with PBS. About 4ml of TPVG (pre-warmed to 37°C) was added over the cells. It was then allowed to act for 3-5 minutes. TPVG was pipetted out and 5ml of 5% MEM was added. The cell clusters were broken by gently pipetting back and forth with Pasteur pipette. Number of cells was counted in a haemocytometer. Sterile Tissue culture bottles were kept properly labelled. About 9ml of growth medium was added to each of the tissue culture bottles. Cell suspension was added to each of the tissue culture bottles based on the cell count. The bottle was shaken gently to allow uniform dispersion of cells. The newly seeded bottles were closed tightly and incubated at 37°C. The cell growth was observed every day. On 4th day the medium was decanted and replenished with 10ml of maintenance medium.

Cell counting

The cell concentration was determined by counting the cells in the haemocytometer. 0.2 ml of the cell suspension was diluted in 0.2 ml of Trypan blue (0.1% Trypan blue). It was mixed well with a pipette and sufficient volume was aspirated and transferred to the haemocytometer immediately. The viable cells (non-viable cells are stained blue and viable cells remain unstained) were counted in each of the four corners of the chambers, omitting cells lying on the top and the left. If cell clumping was observed, it was discarded and original cell suspension was resuspended. The total no. of cells in a suspension was calculated using the following formula.

$$C_1 = T_b \times (T/4) \times 10^4$$

Where,

C₁ - Initial cell concentration / ml

T - Total viable cell count of 4 squares

Tb - Correction for the trypan blue dilution

T/4 - Correction to give mean cells / corner square

10^4 - Conversion factor for counting chamber.

For a 96 well plate cell were seeded at a concentration of 10^4 cells/well

For a 24 well plate cell were seeded at a concentration of 10^5 cells/well

Anticancer assay:

MTT assay: [12]

Non-toxic dilution and anticancer activity of Sample against MCF-7 cell line. Seeded cells at a specific concentration in desired number of wells in a 24 well plate and incubated till cells form a monolayer. Medium was removed from wells for MTT assay. Add 800 μ L of Samples (dilutions made with MEM containing 10%FCS). To each well add 200 μ l of 5mg/ml of MTT (FCS). The plate is incubated overnight at 37°C. At the end of incubation add 1ml of DMSO to each well. Gently pipette back and forth with Pasteur pipette to break the cells and liberate the Formosan crystals. The suspension is transferred into the cuvette of spectrophotometer and OD values are read at 560 nm. Percentage of Cell Viability was calculated using the formula.

Calculation: (OD of Sample/ OD of cell control(y))*100 = % Cell Viability

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability in cytotoxicity and anti-tumor activity assessments.

DNA Fragmentation

0.5ml of cell suspension was dispensed and centrifuged at 200XG for 400°C for 10mins. To the pellet 0.5ml of TTE and vortex it vigorously for the release of fragmented chromatin from nuclei after cell lysis by the presence of triton X-100 in the TTE solution and nuclear disruption by the chelation of Mg^{2+} by EDTA in TTE solution. The suspension was centrifuged at 20000XG at 100°C for 4mins to separate fragmented DNA from intact chromatin. The supernatant was transferred carefully to new tubes labelled as T. To the small pellets 0.5ml of TTE solution was added. 0.5ml of ice-cold 5M NaCl was added and vortex it vigorously, where the addition of salts removes histones from DNA. 0.7ml of ice-cold iso-propanol was added and vortex it vigorously. For precipitation the sample was put in dry ice/ethanol for 1 hour. After precipitation, DNA was recovered by pelleting at 20000XG for 10mins at 4°C. Supernatant was discarded by aspirating or inverting the tubes to remove the fluid drops or carefully for the removal of the fluid adherent to the tubes with a paper towel corner. Pellets were rinsed by adding 0.5-0.7ml of ice-cold 70% ethanol. Again the tubes were centrifuged at 20000XG at 40°C for 10mins. Supernatants were discarded by aspirating or inverting the tubes to remove the fluid drops or carefully for the removal of the fluid adherent to the tubes with a paper towel corner for 30mins. Tubes were dried in the upright position for 3hrs before proceeding. DNA was dissolved in 20-50 μ L of TE solution and the tubes were placed at 37°C.

Cell proliferation assay

Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. After the cell reaches the confluence, the various concentrations of the Samples were added in triplicate and incubated for 24hrs/48 hrs/72 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 μ l/well (5mg/ml) of 0.5% 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1ml of DMSO was added in all the wells. The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC₅₀) was determined graphically. The % cell viability was calculated using the following formula:

Calculation:

$$\% \text{ cell viability} = \frac{\text{A570 of treated cells}}{\text{A570 of control cells}} \times 100$$

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability in cytotoxicity and anti-tumor activity assessments.

RESULTS AND DISCUSSION

Qualitative phytochemical screening result:

Table1 represents the phyto chemical screening in *Syzygium cumini* seed extract of ethyl acetate. Preliminary

phytochemical analysis showed the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, Tannins, proteins and steroids in ethyl acetate and methanol seed extract of *Syzygium cumini*. Azra kamal [13] also reported the presence of preliminary phytochemical analysis of methanolic extract revealed the presence of alkaloids, glycosides, triterpenoids, proteins, saponins, quinines, flavonoids, tannins except carbohydrates in the extracts of *Syzygium cumini* seed.

Phytochemical analysis of ethyl acetate extract of *Syzygium cumini* seed showed that, it contains the presence of alkaloids, flavonoids, tannins, saponins, proteins and potent water soluble components where tannins present in large quantity [14]. Plant steroids are known to be important for their cardiotoxic activities and also possess insecticidal and antimicrobial properties. They are also used in nutrition, herbal medicine and cosmetics [15]. Saponins has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [16, 17]. Saponin is used as mild detergents and in intracellular histochemical staining. It is also used to allow antibody access in intracellular proteins. In medicine, it is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory, weight loss, etc. It is also known to have antifungal properties [18]. Flavonoids were found in the extracts and are potent water soluble antioxidants. Flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus and they showed their anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities [19]. Tannins bind to proline rich protein and interfere with protein synthesis. Tannins were reported to exhibit antiviral, antibacterial and anti-tumour activities. It was also reported that certain tannins were able to inhibit HIV replication selectively and was also used as diuretic [15].

Table 1: Qualitative analysis of Phytochemical screening of Methanol and Ethyl acetate extract of *Syzygium cumini* seeds

S.NO	PHYTOCHEMICAL CONSTITUENTS	ETHYL ACETATE EXTRACT	METHANOLIC EXTRACT
1.	Alkaloids	+	+
2.	Saponins	+	+
3.	Flavonoids	+	+
4.	Tannins	+	+
5.	Anthraquinones	-	-
6.	Quinones	+	+
7.	Reducing sugars	-	-
8.	Steroids	+	+

(+) Presence (-) Absence

Antibacterial activity

Table 2 revealed the antibacterial activity of *Syzygium cumini* seed extract (ethyl acetate) using agar disc diffusion method. Moderate to less activity was monitored against *Escherichia coli*, *Staphylococcus aureus* and *pseudomonas aeruginosa* using ethyl acetate extract of *Syzygium cumini* where the zone of inhibition was high in *Pseudomonas* and *Escherichia coli* when compared to *Staphylococcus aureus*.

Plate 1 showed the diagrammatic representation of antibacterial activity of ethyl acetate extract of *Syzygium cumini* in the concentration of 1000µg, 750µg, 500µg against the bacterial strains *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Plants used in Indian folklore system of medicines have been found active against a wide variety of microorganisms. Many biochemical constituents of plants possess excellent antimicrobial activities. Although the report of the studied plants for the treatment of wound infections is available in literature, we found contradictory and equivocal reports on screening of their extracts against pathogens. Similar result of extracts of *A. nilotica* was found as effective growth controller of *E. coli* and *P. aeruginosa* [20].

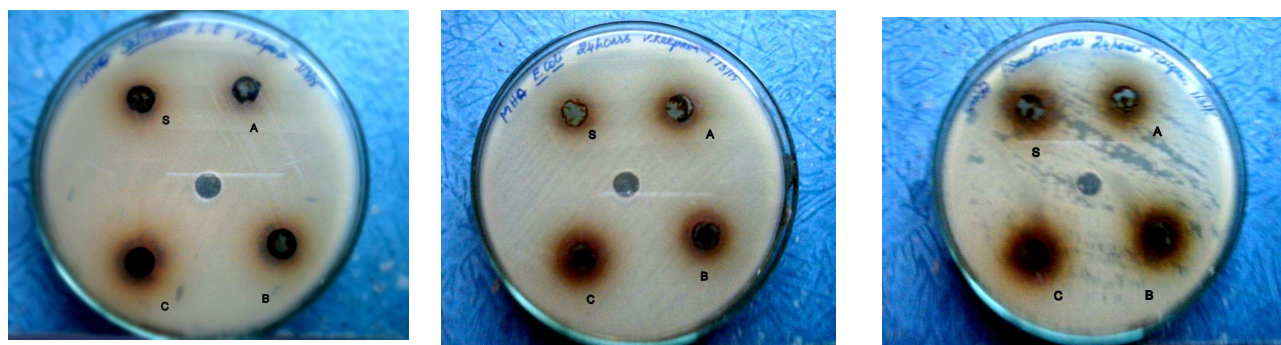
Methanol extract was also reported inhibitory to *B. subtilis*, *P. aeruginosa*, and *S. aureus* but not *E. coli* [21]. Petroleum ether extract was studied for its antibacterial activity by [22] and its high efficacy against *E. coli*, *P. aeruginosa*, and *S. aureus* was concluded. Alcoholic extract of *A. indica* was found inhibitory against the *B. subtilis* and *S. aureus* [23] and, methanol extract was inhibitory against *E. coli*, *S. aureus* and *P. aeruginosa* [21]. However, aqueous extract was inactive against *E. coli*, *S. aureus* and *P. aeruginosa* [24]. Ahmad *et al.* [23] reported that the aqueous extract of *O. Sanctum* was not effective against any bacteria; but the alcoholic extract was highly active against *E.coli*, *S.aureus* and *P. aeruginosa*. A number of explanations can be given for the difference in biological activity reports of some common extracts against same or similar microorganism. In this study all plants and plant parts were collected from western region of India. The activity and quantity of phytochemicals presents in extracts can be varying depending upon geographical locations of plant cultivation [25].

In the findings, there were marked differences in the activities of some extracts in two antimicrobial testing methods. The variation in results during the antimicrobial efficacy in different testing methods of a compound transpires

because of effect of medium and supplements [26], temperature and other inoculation conditions [27] molecular weight and diffusion rate of compound through medium [28, 25].

Table 2 : The antibacterial activity of *Syzygium cumini* seed extract (Ethyl acetate) using agar disc diffusion method

Organisms	Zone of inhibition (mm)			Antibiotic (1mg/ml)	DMSO (20µl)
	Concentration (µg/ml)				
	1000	750	500		
<i>Staphylococcus aureus</i>	9mm	6mm	5mm	8mm	-
<i>Escherichia coli</i>	12mm	9mm	5mm	6mm	-
<i>p.aeruginosa</i>	12mm	9mm	5mm	9mm	-



Escherichia coli(E.coli)

Staphylococcus aureus

Pseudomonas aeruginosa

S – STANDARD ; A -500 µg, B -750 µg, C-1000 µg

Plate1: Anti microbial activity of *Syzygium cumini* seeds

Antifungal activity

The crude extract of *Syzygium cumini* is studied for antifungal activity. Antifungal activity of seed extract of *Syzygium cumini* showed moderate activity against *Candida albicans* and *Aspergillus niger*. The standard for antifungal activity was Amphotericin B. **Table3:** Antifungal activity of *Syzygium cumini* seed in the ethyl acetate crude extract against fungal species using by agar diffusion assay. **Plate 2** showed the diagrammatic representation of the antifungal activity of ethyl acetate seed extract of *Syzygium cumini* in the concentration of 1000µg, 750µg, 500µg against the fungal strains *candida albicans* and *Aspergillus niger*.

The hydro alcohol extract of *Valeriana jatamansi*, showed maximum antifungal activity against *aspergillus niger* and *candida albicans*.

Al-Bayati and Mola [29] Demonstrated that ethanolic extracts of medicinal herbs inhibit growth of *Candida albicans*. The present study showed that similar extracts from *Elettaria cardamomum* and *Aloe vera* at the concentrations of 400, 200, 150, 100, 50, and 25 mg/ml for each extract have promising antifungal activity against six isolates of *Candida*, while ethanolic extract of *Thymus vulgaris* at the same concentrations produced no effect on the growth of this yeast, and also the solvent control (96% ethanol) showed very limited anti candidal activity. Agarry *et al.* [30] had been found that the growth of *Candida albicans* was also inhibited by *Aloevera* leaf extract but was not affected by the gel, this positive effect could be related to its active constituent mainly the anthraquinone glycoside that present in the leaf juice (latex) or extract but not in the gel. Making this study resembling ours in their positive results coming from the use of ethanolic extract of *Aloe vera*. Sehgal *et al.* [31] in a study done on the efficacy of *Calotropis procera* latex on *Candida* growth in comparison to three standard antifungals drugs were found that nystatin was more effective when compared with the used clotrimazole and griseofulvin. Clotrimazole is belonging toazole antifungal agent, it interact synergically *in vitro* with zeamatin as shown in systemic therapy against murine candidiasis The antifungal effect of *S. aromaticum* and *C. zeylanicum* found on *Aspergillus spp.* and *Penicillium spp.* was also reported earlier [32-34] Presence of ajoene and alliicin in *A. sativum* might be the reason for their complete inhibition of *A. niger* [35, 36]

Amadioha [37] investigate the aqueous extracts of other spices like *Murrayakoeningii*, *Zingiber officinale*, and *Allium cepa* were not proved to be effective against the growth of *A. niger*. The ineffectiveness of these spices on *A. niger* might be due to insolubility of their active compounds in water [38, 37].

Table 3: Antifungal activity of *Syzygium cumini* seed in the ethyl acetate crude extract against fungal species tested by agar diffusion assay

Organisms	Zone of inhibition (mm)			Antibiotic (1mg/ml)	DMSO (20µl)
	Concentration (µg/ml)				
	1000	750	500		
<i>Candida albicans</i>	6mm	5mm	3mm	9mm	-
<i>Aspergillus niger</i>	4mm	2mm	1mm	6mm	-

*Candida albicans**Aspergillus niger***Plate 2: Antifungal activity of *Syzygium cumini* seeds****Antioxidant assay**

Complex antioxidant systems are very important for protecting cellular membranes and organelles from the damaging effects of active oxygen species. **Table 4:** represents the antioxidant activity of *Syzygium cumini* seed in ethyl acetate and methanolic extract tested by DPPH radical scavenging assay. From the results it was known that ethyl acetate seed extract of *Syzygium cumini* has high antioxidant activity when compared to ethanol and methanolic seed extract of *Syzygium cumini*.

Benherlal and Arumugan [39] evaluated the antioxidant effects of the ethanolic extract of the fruit pulp, kernel and seed coat in various *invitro* assays (DPPH•, OH•, O₂•- and lipid peroxidation) with gallic acid, quercetin and trolox as reference molecules. In the DPPH scavenging assay and lipid peroxidation assays the kernel extract was better than the seed coat and pulp extract, but less than the reference molecules. However in the superoxide radical scavenging activity the kernel extract was six times more effective than trolox and three times than catechin. In hydroxyl radical scavenging assay, the kernel extract was comparable to the effect of catechin [39]. The hydroethanolic extract of the seed [40], methanolic extracts of stem [41], anthocyanin-rich fruit peel extract [42] and the methanolic extract of the leaves [41, 43] are observed to be free radical scavengers in the DPPH• scavenging assay. The hydrolysable and condensed tannins in the fruit are also reported to possess antioxidant activity in the DPPH radical scavenging and FRAP assays [44].

The organic extract of the leaf (methanol dichloro methane extract) as well as the hydroethanolic extract of the seed is reported to be a scavenger of nitric oxide *in vitro* [45].

Ruan *et al.* [46] subjected the methanolic extract of leaf to various fractions (viz water, ethyl acetate, chloroform and *n*-hexane) and studied their free radical scavenging effects in the DPPH and FRAP assays. It was observed that in the DPPH assay the efficacy was as follows ethyl acetate fraction ≈ methanolic extract > chloroform fraction ≈ water fraction > *n*-hexane. In the FRAP assays similar observations were observed and except for the hexane fraction, all other fractions showed high ferric reducing power at high concentrations [46]. The fruit skin of Jamun possess antioxidant effects as confirmed by results from the hydroxyl radical-scavenging assay, superoxide radical-scavenging assay, DPPH radical scavenging assay and lipid peroxidation [47]. The anthocyanin-rich fruit peel extract is also observed to be an effective reducing agent [42]. Recently, Bajpai *et al.*, have also observed that the hydromethanolic extract of the Jamun seed was effective in scavenging (90.6%) free radicals as evaluated in the auto-oxidation of β-carotene and linoleic acid assay and was due to the presence of high total phenolic content in the extract [48].

Table 4: The antioxidant activity of *Syzygium cumini* seed in ethyl acetate, ethanol and methanolic extract by using DPPH radical scavenging assay

Seed extract	Volume of sample (μ l)	Volume of methanol (ml)	Volume of DPPH dye (μ l)	Incubation	Absorbance at 517 (nm)	%of scavenging free radicals
Ethyl acetate seed extract	100	3.7	200	Kept in dark place for 30 minutes	0.06	86.11%
Ethanolic seed extract	100	3.7	200		0.08	80.55%
Methanolic seed extract	100	3.7	200		0.07	81.39%

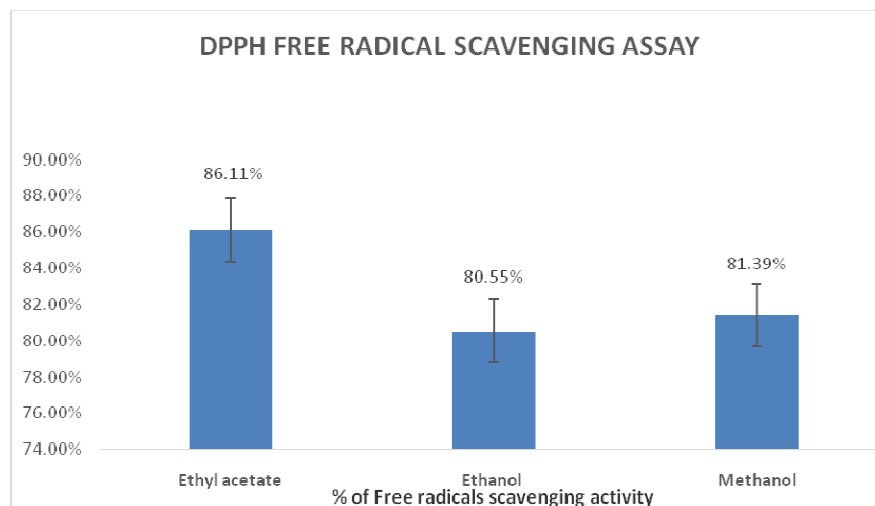
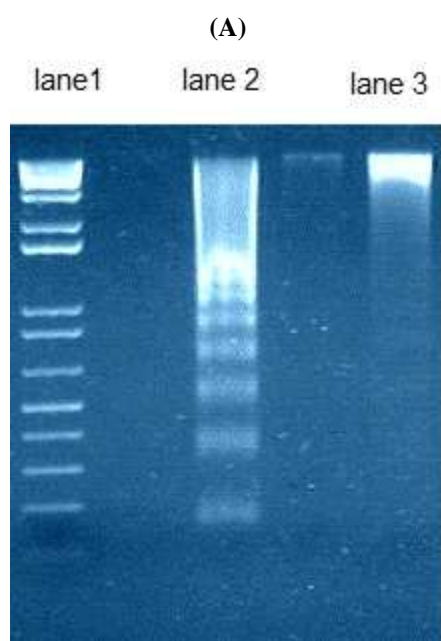
**Figure 1:** Antioxidant activity of methanol, ethanol and ethyl acetate extract of *Syzygium cumini***DNA fragmentation assay**

Plate 3 refers the DNA fragmentation results by *Syzygium cumini* seed against MCF-7 cell lines. The DNA from MCF-7 cell line treated with ethyl acetate and methanol extract where it reports the apoptosis. lane 1 aliquot with 1KB ladder DNA, lane 2 aliquot with MCF-7 DNA pellets with ethyl acetate extract, lane 3 aliquot with MCF-7 DNA pellets with methanolic extract. The highest damage of DNA was noticed in ethyl acetate treated cell extract treated cells which showed many bands in the DNA fragmentation analysis. One of the important hallmarks of apoptosis is DNA fragmentation. As shown in agarose gel electrophoresis, increased DNA fragmentation was apparent in treated MDA-MB-231 and MCF-7 cells as compared to the untreated cells. Flow cytometric analysis showed that *A. Precatorius* has a pro-apoptotic activity on both MDA-MB-231 and MCF-7 cells. A significant increase sub G0/G1 population of cells was analyzed from (9.14% to 42.18%) in MDA-MB-231 cells and (16.6% to 39.18%) in MCF-7 cells after 24 h treatment, as compared to control cells.

**Lane 1:** 1KB ladder; **Lane 2:** DNA from MCF-7 cells with ethyl acetate extract; **Lane 3:** DNA from MCF-7 cells with methanolic extract

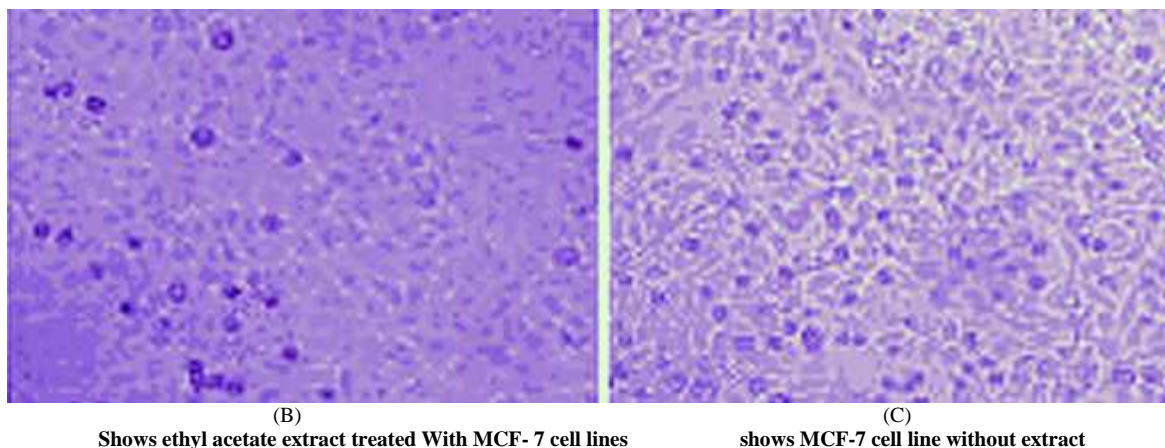


Plate 3: DNA fragmentation in *Syzygium cumini*

This is an important indicator of apoptosis. This study provides an important basis for further investigation into the isolation, identification, characterization and molecular mechanism behind the specific cytotoxic activity [49].

Anticancer assay

Cytotoxicity assay

For cytotoxicity, different concentrations of ethyl acetate extract and methanol extract of *Syzygium cumini* were tested against MCF-7 cell line. MTT assay was carried out in 24 well plates. It was concluded that ethyl acetate seed extract shows less viability of cells when compared to methanolic seed extract of *Syzygium cumini*. **Table 5 and 6** reported the anticancer activity of *Syzygium cumini* in the ethyl acetate and methanolic seed extract by using MTT assay. From the results the ethyl acetate seed extract of *Syzygium cumini* has high anticancer activity than the methanolic extract. **Figure 2** showed the graphical representation of anticancer activity of ethyl acetate and methanolic extract of *Syzygium cumini*. MCF-7 cell lines grown in DMEM, when subjected to different concentrations of *Rubia cordifolia* extract resulted in 46.19 % inhibition. Similarly *Plumbagozeylanica* resulted in 50.23% of MCF-7 cell death whereas *Calophyllum inophyllum* displayed weak inhibition of 31.25%. On the other hand, comparison with Tamoxifen showed that 85.4% MCF-7 cell line inhibition at the same tested dose. Cytotoxicity was determined using the standard colorimetric MTT assay. Based on this assay out of the selected medicinal plants, only *A. precatorius* showed significant activity on growth and proliferation of breast cancer cells. Previous preliminary studies also reported significant cytotoxic activities of various plant crude extracts on various types of breast cancer cell lines [49].

Table 5: The anticancer activity of *Syzygium cumini* in the methanolic seed extract tested by using MTT assay

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.12	12.06
2	500	1:1	0.17	25.86
3	250	1:2	0.24	36.20
4	125	1:4	0.30	50.00
5	62.5	1:8	0.37	58.62
6	31.2	1:16	0.42	67.24
7	15.6	1:32	0.45	77.58
8	7.8	1:64	0.49	82.75
9	Cell control	-	0.58	100

Table 6: The anticancer activity of *Syzygium cumini* in the ethyl acetate seed extract by using MTT assay

S.No	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.05	9.25
2	500	1:1	0.10	18.51
3	250	1:2	0.13	24.07
4	125	1:4	0.17	31.48
5	62.5	1:8	0.23	42.59
6	31.2	1:16	0.29	53.78
7	15.6	1:32	0.37	68.51
8	7.8	1:64	0.40	74.07
9	Cell control	-	0.54	100

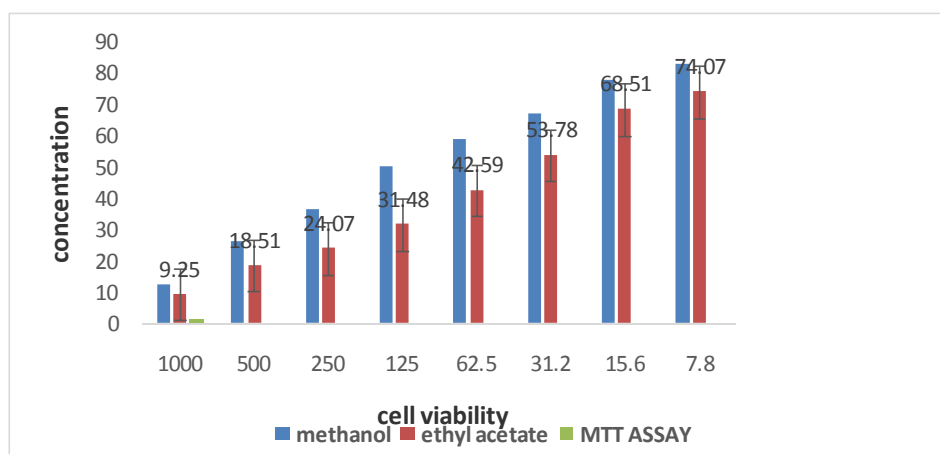


Figure 2:Anticancer activity of ethyl acetate and methanolic extract of *Syzygium cumini*

Different dilutions of extracts were treated and IC₅₀ values were calculated. In our screening program, we adopted the criteria of the American National Cancer Institute to consider a crude extract promising for further purification based on the IC₅₀ values lower than 30 g/mL in order to discover and develop potential anticancer natural compounds [50, 51]. Cytotoxicity screening models provide important preliminary data to help selecting plant extracts with potential antineoplastic properties for future work [52]. It is of interest that the extract of the plants showed cytotoxicity against cancer cell line, and, if this also occurs *in vivo*, the use of these plants by traditional healer for the treatment of cancer patients would have some scientific support. Several plant species rich in flavonoids are reported having disease preventive and therapeutic properties. This observation is of particular importance since flavonoids are ingredients of many vegetables and fruits and the association of vegetable and fruit consumption with reduced cancer risk has been reported [53, 54]. Cytotoxic activity recorded in the present study is in accordance with this finding, since the phytochemical evaluation indicated the presence of flavonoids in all of the three plant species with promising activity. High contents of quercetin, myricetin and kaempferol were identified in *S. grandiflora* leaf extracts [55]. A novel protein fraction was isolated from the flower of *S. grandiflora* which showed potential anticancer and chemo preventive efficacy [56]. Recently nine flavonoids, artocarpin, cudraflavone C, 6-prenylapigenin, kuwanon C, norartocarpin, albanin A, cudraflavone B, brosimone I and artocarpanone were identified from the methanol extract of the wood of *A. heterophyllum* which showed *in vitro* cytotoxic activity against B16 melanoma cells [57]. The cytotoxic activities of active plants are probably due to presence of flavonoids.

Cell proliferation

Table 7 revealed anticancer activity of *Syzygium cumini* in ethyl acetate crude extract determined by cell count. From the results the ethyl acetate crude extract of *Syzygiumcumini* has high inhibition of cell proliferation than the ethyl acetate extract. Antiproliferative effect of Green tea extract was evaluated on MCF-7 cell line. MCF-7 were cultured in DMEM medium and incubated with different concentrations (18.75, 37.5, 75, 150 and 300 g/ml) of methanol extract of Green tea. Cell viability was assessed by MTT assay. Green tea decreased cell viability in malignant cells in a concentration dependent manner. The IC₅₀ value of green tea extract was found to be 111.9 g/ml. It may be concluded that green tea could cause cell death in HeLa cells and can be considered as a promising Antiproliferative agent against cervical carcinogenesis [58].

Preliminary qualitative phytochemical analysis of ethyl acetate and methanol extracts of *Syzygium cumini* seeds have shown the presence of Alkaloids, Steroids, Flavonoids, Saponins, Quinones, tannins and Proteins. The ethyl acetate extract of *Syzygium cumini* seeds has shown moderate to less activity against 3 pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, Where zone of inhibition was high in *Pseudomonas aeruginosa* and *Escherichia coli*. The ethyl acetate extract of *Syzygium cumini* seeds has high significant inhibitory activity against 2 fungi's such as *Candida albicans* and *Aspergillus niger*. The ethyl acetate, methanol and ethanolic extracts of *Syzygium cumini* seeds showed significant antioxidant activity which helps in scavenging the free radicals against MCF-7 breast cancer cells. Ethyl acetate extract of *Syzygiumcumini* has shown the maximum scavenging activity when compared to other two extracts.

The ethyl acetate and methanolic extracts of *Syzygium cumini* showed significant anticancer activity against MCF-7 breast cancer cells. Viability of cells was very less in ethyl acetate extract of *Syzygium cumini* when compared to methanolic extract of *Syzygium cumini*. It was concluded that ethyl acetate extract posses higher anticancer activity

when compared to methanolic extract of *Syzygium cumini* The cell proliferation and cell viability of MCF-7 breast cancer cells was highly inhibited by the ethyl acetate extract of *Syzygium cumini* seeds. Finally it was concluded that the phyto constituents present in the *Syzygium cumini* seeds could provide potential bioactive compounds for the development of new leads to cancer diseases.

Table 7: Cell proliferation of *Syzygium cumini* in ethyl acetate crude extract tested by

S.No	Concentration (µg/ml)	Cell Viability (%)		
		24 hrs	48 hrs	72 hrs
1	1000	26.666	8.888	6.666
2	500	24.444	6.666	4.444
3	250	11.111	4.444	2.222
4	125	6.666	2.222	0.00
5	62.5	4.444	0.00	0.00
6	31.2	2.222	0.00	0.00
7	15.6	0.00	0.00	0.00
8	7.8	0.00	0.00	0.00

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