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Antibacterial and Anticancer Activity of Bioactive Fraction of *Syzygium cumini* L. Seeds

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***Syzygium cumini* L. better known as Jamun belonging to the family Myrtaceae is identified to have antidiabetic, anti-inflammatory, anti-pyretic and anti-oxidant activities. Anticancer activity of *S. cumini* L. fruits has been demonstrated. However, anticancer activity of *S. cumini* seeds on various types of human cancers has not been explored much. The methanol fraction of ethanol extract from the seeds of *S. cumini* was found to have significant antibacterial activity. This bioactive fraction was further tested positive for its anticancer activity on various types of human cancer cell lines indicating its potency. Structural characterization of the bioactive fraction was achieved using analysis of high performance liquid chromatography, ultra violet and infra red spectrum.**

Key words: *Syzygium cumini* L., antibacterial activity, cytotoxic activity, structural characterization

INTRODUCTION

The Myrtaceae is a large family of plants consisting of trees and shrubs found in the tropics and subtropics and comprises about 150 genera and 3,600 species (Cronquist 1981). *Syzygium cumini* L. better known as Jamun is an important member of this family widely distributed all over the Indian sub-continent (British Herbal Pharmacopeia 1976). The fruits and seeds of *S. cumini* are used to treat diabetes mellitus for several centuries in folklore medicine of south Asia. The seeds are used as astringent and diuretic (Bhatia & Bajaj 2005). They have hypoglycaemic (Chopra *et al*. 1958; Mahapatra *et al*. 1985; Stanely *et al*. 1998a), anti-inflammatory (Chaudhary *et al*. 1990), antipyretic (Ghosh *et al*. 1985), psychopharmacological (Chakraborty *et al*. 1985), hypolipidaemic (Stanely & Menon 1997), and antioxidant (Stanely & Menon 1998b; Banerjee *et al*. 2005; Benherlal & Arumughan 2007; Bushra *et al*. 2007) activities. Craveiro *et al*. (1983), studied the essential oils of leaves, stems, and fruits of *S. cumini* and their antibacterial activity was reported by Shafi *et al*. (2002).

Cancer is a public health problem worldwide. According to WHO, 20 million people in the world suffer from cancer, a figure projected to rise to 30 million within 20 years (WHO 2004). The search for anticancer agents from plants dates back to 1947, when the cytotoxic properties of podophyllotoxin from *Podophyllum peltatum* (Berberidaceae) were detected (Kelly & Hartwell 1954). The discovery of the antileukemic properties of vinblastine and vincristine from *Cataranthus roseus* (Apocynaceae) soon followed (Noble *et al*. 1958) and gave the impulse for wider ranging investigations of plant extracts and plant-derived compounds for possible anticancer activity. In the case of human cancers, thus far, nine plant-derived compounds have been approved for clinical use in the United States. They are vinblastine, vincristine, the campothecin derivatives-topotecan and irinotecan, etoposide and paclitaxel. A number of promising agents such as roscovitine, betulinic acid and silvestrol are in clinical or preclinical development (Shoeb 2006).

Few reports have indicated potential of *S. cumini* fruits to combat cancer. Nazif (2007) isolated four anthocyanins from acidic alcoholic extract of *S. cumini* fruits in Egypt and the crude extract was found to exhibit potent cytotoxic activity on several types of human cancer cell lines. Barh and Viswanathan (2008) showed that the crude extract of *S. cumini* fruits inhibited growth and induced apoptosis in cervical cancer cell lines HeLa and SiHa in a dose and time-dependent manner. There is no report available on anticancer activity of extracts of *S. cumini* seeds. So the present study was undertaken to isolate, characterize a bioactive fraction from *S. cumini* seeds and evaluate its anticancer activity on human cancer cell lines in vitro.

MATERIALS AND METHODS

Collection of Plant Material. *Syzygium cumini* L. (Jamun) fruits were collected fresh from Keshav Shristi, Bhayandar, brought to the laboratory and washed with distilled water. The plant was identified and authenticated at the Blatter Herbarium St. Xavier's College, Mumbai-400 001, India, with specimen no. 1848 of G. L. Shah. The outer covering of the fruit was removed; seed was taken and

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dried in oven at a constant temperature. The dried seeds were ground in electrical mill and the powdered seeds were kept separately in plastic containers in dark until used.

Preparation of Ethanolic Seed Extract of *Syzygium cumini* L. A suspension of 800 g of seed powder in 1,000 ml of ethanol was stirred manually for half an hour and the resultant mixture was kept in the refrigerator at 4 °C overnight. The suspension was then centrifuged for 5 minutes at 6,000 rpm. The supernatant was concentrated in a rotary evaporator. The process was repeated for seven days to get 20 g ethanol extract.

This resultant alcoholic extract obtained was subjected to solvent extraction method using petroleum ether, chloroform and ethyl acetate. The ethyl acetate fraction was found to be bioactive using bioassay and thus selected for further studies. This bioactive fraction was subjected to column chromatography to obtain a methanolic fraction which was further purified using preparative thin layer chromatography using solvent system chloroform:ethyl acetate:methanol (30:50:20) to isolate a single compound.

Evaluation of Antibacterial Activity (Bioassay). The isolated compound was tested against four microorganisms by agar cup method (Spooner & Skyes 1972). Bioassay was done using agar cup method using two gram positive and two negative test organisms. Agar wells were made using sterile cork borer of diameter 8 mm. All bioassays were carried out in triplicate and average values were taken.

Evaluation of Anticancer Activity Cell lines and Culture Maintenance Conditions. CellTiter96® AQueous non-radioactive MTS reagent was purchased from Promega Co. (WI, USA). Fetal bovine serum (FBS) was from Hyclone (Logan, UT, USA). All other chemicals and media were purchased from Sigma. The human cancer cell lines MCF7 (breast adenocarcinoma), A2780 (ovarian adenocarcinoma), PC-3 (prostate carcinoma), H460 (non-small cell lung carcinoma) were purchased from American Type Culture Collection (MD, USA). All the cell lines were grown in RPMI-1640 culture medium, supplemented with 10% heat inactivated FBS, 2% L-glutamine (200 mmol/l) and 1% penicillin-streptomycin. Cells were grown as a monolayer culture in T-75 flasks at 37 °C in a humidified 5% CO_2 incubator.

***In Vitro* Cytotoxicity Assay.** When 80% confluence was reached in T-75 flasks, cells were harvested with 0.025% trypsin/EDTA, washed with PBS and counted using trypan blue dye exclusion method. Each cell line was seeded into 96-well flat bottom microtiter plates at a density of 3,000 cells/well and left to attach to the plates for 24 h. Next day, 20 µl of culture medium or medium containing the test compound was added to the wells. Each concentration was plated in triplicate. The isolated compound from the seeds of *S. cumini* L. was dissolved in dimethylsulfoxide (DMSO) and applied in five concentrations (300, 100, 30, 10, 3 µg/ml). Flavopiridol (positive control) was applied in four concentrations (1, 0.3, 0.1, and 0.03 µmol/l). Following 48 h of drug exposure, the effect on the cell viability was measured using MTS cell proliferation assay (Cory *et al*. 1991). 20 µl of CellTiter96® Aqueous non-radioactive MTS reagent was added to each well. Cells were incubated further to allow for color development before the absorbance values were read at 490 nm using a microplate reader. A blank control was included by mixing media and MTS reagent absence of cells. The highest DMSO concentration was 0.5%. At this concentration, DMSO did not alter cell viability (data not shown).

Structural Characterization. Structural Characterization of the bioactive fraction was done with the help of UV spectrophotometer (Pharma Spec by Schimadzu) to find out the wavelength of maximum absorption. It was followed by HPLC analysis (Model No. 1100 by Agilent with binary pump and VWD detector) to check the purity of the isolated fraction. Then an FTIR spectrum (Perkin Elmer) was studied to find out the functional groups present in the isolated fraction. Melting point was also recorded by capillary method.

RESULTS

Purified methanol fraction from ethanol extract of the seed of S. *cumini* L. exhibited significant antibacterial activity against all the test microorganisms (Table 1). Highest zone of inhibition of 20 mm was observed against *E. coli* while *Pseudomonas aeruginosa* was found to be moderately sensitive as it indicated an inhibition zone of 11 mm. Overall the isolated compound was found to be active against both gram positive as well as gram negative organisms suggesting its broad range of antibacterial activity.

The cytotoxic activity of the seed extract of *S. cumini* L. on various cancer cell lines was examined by MTS assay. The results of the cytotoxicity evaluation exposure time 48 h are summarized in Table 2. Growth stimulation/ inhibition was calculated as treated/control × 100 (%T/C). Cytotoxicity curves (Figure 1) were obtained by plotting percentage of viability against different concentrations of the extract. All results were expressed as IC_{50} values, the concentration of the extract that inhibited cell proliferation up to 50% of the negative control. A2780

Table 1. Antibacterial activity of isolated compound from *S. cumini* L. seeds

Test organism	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*
Zone of inhibition (mm)	20	11	17	15

Table 2. Mean IC_{50} values of isolated compound from *S. cumini* L. seeds in human cancer cell lines

Cell line	Mean IC_{50} (µg/ml)	
	S. cumini seed extract	Flavopiridol
A2780	49	0.06
MCF7	110	0.06
PC-3	140	0.08
H460	165	0.07

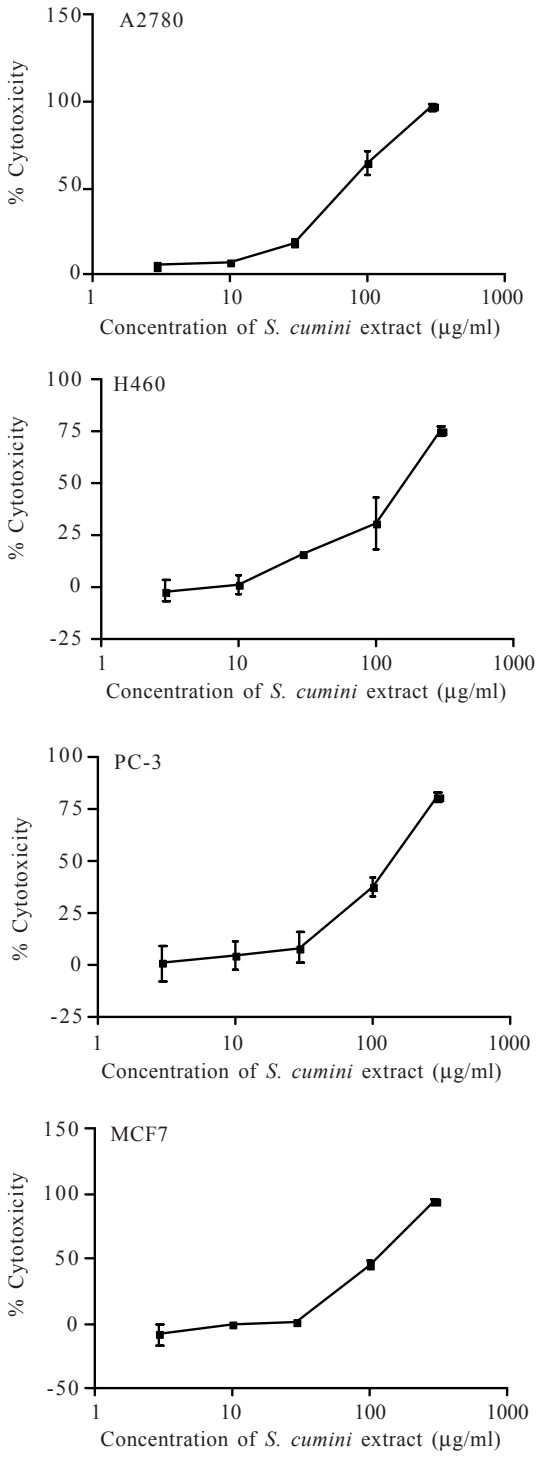

Figure 1. Growth inhibitory effect of isolated compound from *S. cumini* L. seeds on various cancer cell line.

(ovarian cancer) cell line was found to be most sensitive with IC_{50} value of 49 µg/ml while H460 (non small cell lung carcinoma) was least sensitive (IC_{50} value of 165 µg/ml). Flavopiridol (positive control) showed very low IC_{50} values (0.06-0.08 µg/ml) on all the cell lines.

Ultra violet spectrum (Figure 2) showed a peak at 217 nm indicating presence of C=C double bond while a peak at 270 nm indicated presence of carbonyl (>C=O) group along with conjugation. HPLC analysis confirmed the

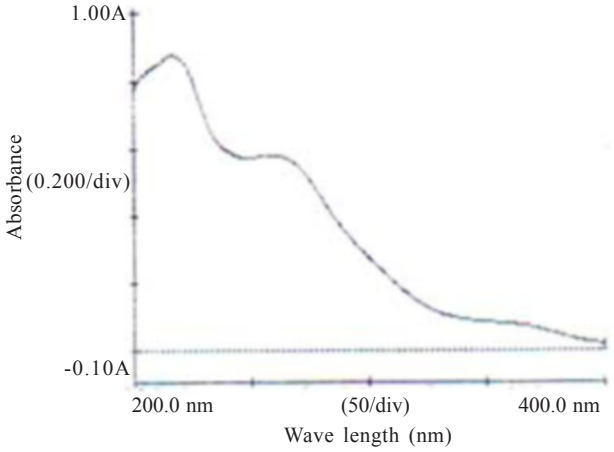

Figure 2. UV spectrum of isolated compound from *S. cumini* L. seeds.

purity of isolated compound whose percentage area was found to be 96.67% and peak height of 10143.55 µAU having a retention time of 17.441 mins (Figure 3). FTIR spectrum (Figure 4) showed peaks at 3367 cm^{-1} and 3288 cm^{-1} indicating the presence of –OH (hydroxyl) group whereas a peak at 1702 cm^{-1} indicated presence of >C=O (carbonyl) group, three peaks at 1617, 1541, and 1449 cm^{-1} indicated presence of aromatic C-C stretching while peak at 1339 cm^{-1} suggested presence of aliphatic C-H stretching. Finally peaks at 1245 and 1025 cm^{-1} indicated presence of aliphatic C-C stretching in the bioactive fraction. Melting point was found to be above 250 °C.

DISCUSSION

Bioassay studies clearly indicated that the purified methanol fraction has antibacterial activity against both gram positive as well as negative bacteria and therefore in future, it could be further purified and explored for producing antibacterial substances from this easily available and widely distributed cheaper plant. This could be a reason of usage of *S. cumini* seeds in traditional Ayurvedic and Homeopathic medicines. Earlier, Karthic *et al*. (2008) showed that aqueous extract of *S. cumini* seeds showed inhibition against porcine pancreatic α-amylase. Constituents of *S. cumini* seeds were found out to be fatty oils, tannins which include ellagic acid, ellagitannins, gallic acid, corilagen, phenolic componets such as ferulic acid, quercetin, veratrole, caffeic acid (EMEA 1999). Among these, ferulic, caffeic, gallic acids were tested for their potential anti proliferative and cytotoxic properties in human breast cancer cell line (MCF-7) as well as on a spontaneous mammary adenocarcinoma tumor. As a single agent, caffeic acid showed substantial growth inhibitory activity (Indap *et al*. 2008). Caffeic acid and its derivatives have been shown to be effective against HL-60 (human leukemia) cells (Chen *et al*. 1996). Flavonoid quercetin has been demonstrated to possess anticancer activity against different types of cancer cells (El Attar & Virji 1999; Aalinkeel *et al.* 2008; Zamin *et al*. 2009; Du *et al*. 2009; Shan *et al*. 2009; Zhang

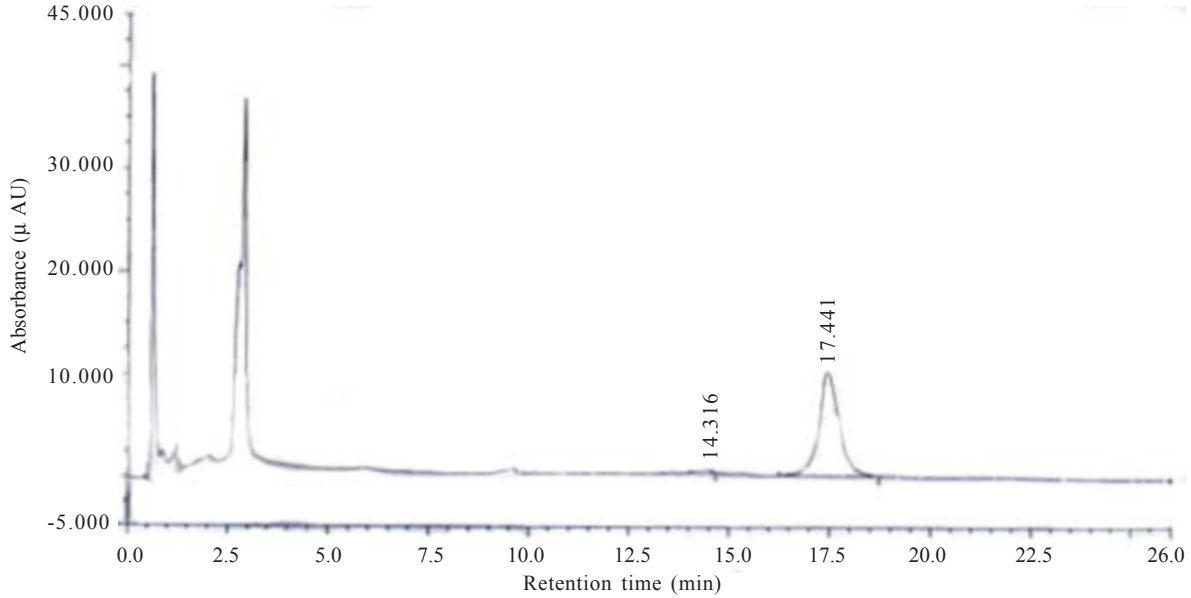

Figure 3. HPLC spectrum of isolated compound from *S. cumini* L. seeds. λ = 254 nm.

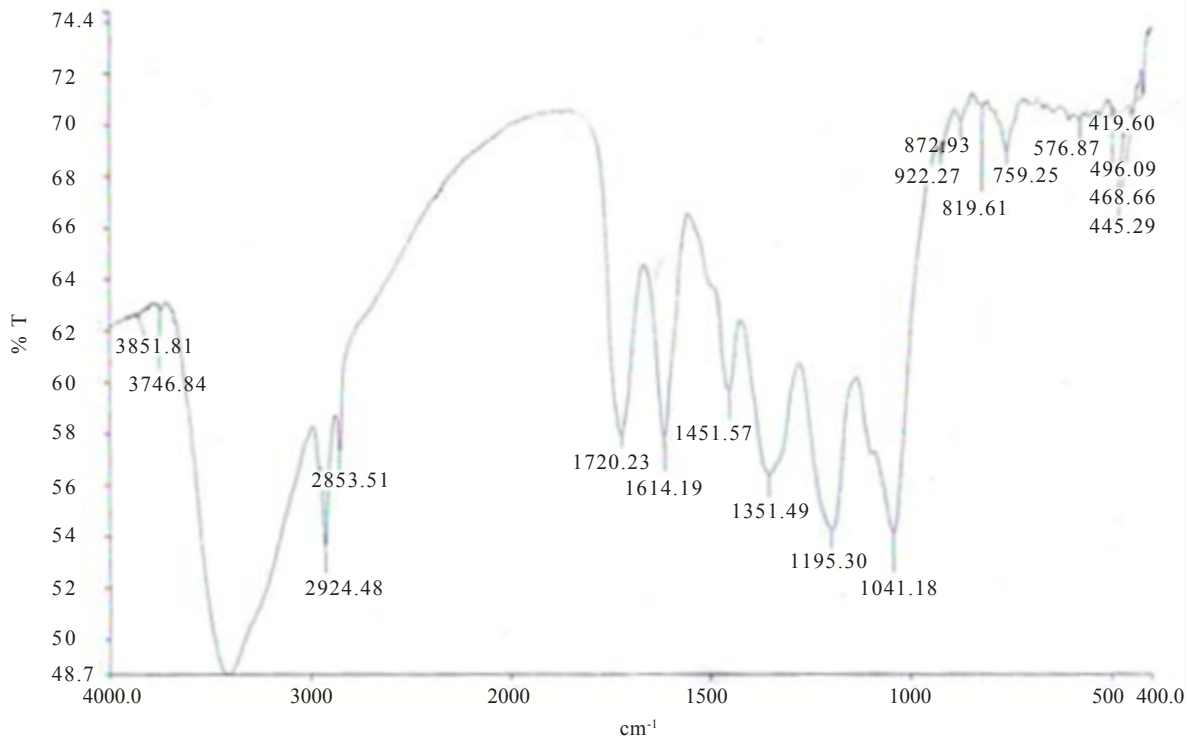

Figure 4. FTIR spectrum of isolated compound from *S. cumini* L. seeds.

& Zhang 2009). Ellagic acid has been demonstrated to have anticancer activity against several types of cancer such as skin, pancreas, colon and esophegal cancer (Edderkaoui *et al*. 2008). Thus, the anticancer activity of *S. cumini* L. seed extract could be attributed to these constituents. This is the first ever report of its kind which reports cytotoxic activity of bioactive extracts prepared from seeds of *S. cumini* L. which are considered to be a waste – useless product and often discarded after consuming the fruit pulp. More studies are therefore warranted to isolate and characterize such bioactive fractions from this plant.

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