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PHYTOCHEMICAL ANALYSES, ANTIBACTERIAL, *IN VITRO* ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF ETHANOLIC EXTRACT OF *SYZYGIUM CUMINI* (L.) SEED EXTRACT

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ABSTRACT

Phytochemical constituents present in the aqueous and ethanolic extract of *Syzygium cumini* seeds were investigated which were found to be responsible for its medicinal efficacy. The major phytoconstituents present in *S. cumini* seeds were further determined by GCMS analysis which showed the presence of polyphenols, sesquiterpenes, n-alkanes. The antibacterial activity of ethanolic extract of *S. cumini* seeds were tested against common human pathogens by agar well diffusion method and minimum inhibitory concentration required to inhibit the growth of various pathogens were also evaluated. The seed extract was found to have high antibacterial activity. The total polyphenolic content of the ethanolic extract of *S. cumini* seed was determined by Folin-Ciocalteau method. The antioxidant activity of *S. cumini* seed ethanolic extract was evaluated by DPPH free radical scavenging assay, reducing power assay and total antioxidant capacity. *S. cumini* seed extract was found to have very high and potent antioxidant activity and might be due to the presence of high phenolic components in seed. The cytotoxicity assay of *S. cumini* seed ethanolic extract was carried out by trypan blue dye exclusion technique where the seed extract showed potential cytotoxic activity against cervical cancer cells.

INTRODUCTION: From ancient times, medicinal herbs have been use in one form or another, under indigenous systems of medicine like Ayurveda, Sidha and Unani [1]. Herbal medicines are promising choice over modern synthetic drugs as they show minimum or no side effects and are considered to be safe [2].

According to WHO, 80% of the world's inhabitants problem should treated by medicinal herbal drug for their primary health care [1]. There are various medicinal plants which have significant medicinal values and are widely used in folklore medicine. The literature reveals that flavonoids, alkaloids, terpenes of plant derived products have received considerable attention in recent years due to their diverse pharmacological properties including cytotoxicity and cancer chemopreventive effects [3].

There are several factors which are responsible for causing various diseases and complications of the human health. In this present study, we have focused on the major two issues related to their complications on human health, multidrug resistant microorganisms and oxidative stress.

The present problem in the pharmaceutical industry is the wide prevalence of the multidrug resistant bacteria. The problem of microbial resistance of growing and the outlook for the use of antimicrobial drugs in the future is still uncertain [4].

So, there is a need to evaluate the plant as the source of potential chemotherapeutic agent, antimicrobial agent and their ethno medicinal use [5].

Another factor that inevitably affects the human health is the generation of free radicals. Free radicals such as Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions [6] and can react with and damage complex cellular molecules such as fats, proteins, or DNA [7]. In low/moderate concentrations free radicals are involved in normal physiological functions but excess production of free radicals or decrease in antioxidant level leads to oxidative stress [8].

Oxidative stress is apparent in pathology associated with aging and many age-related chronic diseases, including atherosclerosis, diabetes mellitus, rheumatoid arthritis, and neurodegenerative diseases [9]. There are synthetic antioxidants such as butylated hydoxyanisole (BHA), butylated hydoxytoluene (BHT), Propylgallate (PG), and test butylated hydroquinone, but owing to their side effects such as liver damage and carcinogenesis [10, 11]. Therefore, there is a need for isolation and characterization of natural antioxidant having less or no side effects, for use in foods or medicinal materials to replace synthetic antioxidants [12].

Plants may possess pharmacological properties, but they may also be toxic or mutagenic. The toxic effects of most widely used medicinal plants are not well documented in the literature although one might expect plants used in traditional medicine over a long period to be safe [13]. The severity of toxicity produced by any chemical is directly proportional to the concentration and the exposure time and this depends on the developmental stage of an organism and its physiological status [14].

Syzygium cumini (L.) is one such traditional medicinal plant of Indian origin, belonging to family Myrtaceae [2, 4, 15]. It is commonly known as Jamun (Hindi) [2], Naaval (Tamil) [15], Java plum, Black plum, Jambul and Indian Blackberry [16, 17]. The medicinal value of *S. cumini* lies in its leaves, fruits, seed and bark. The seeds of *S. cumini* are widely considered to have anti-diabetic properties [2, 4].

The fruits [18] and leaves [19] of *S. cumini* have unique anti-oxidant activity. Earlier studies showed that the seeds have significant anti-inflammatory [20], anticancerous [3] and central nervous system [15] activities. In this present study, the ethanolic extract of *S. cumini* seed in regard to its antibacterial, *in vitro* antioxidant and *in vitro* cytotoxic activities were evaluated.

MATERIALS AND METHODS:

Chemicals and reagents: Nutrient broth, nutrient agar, 2,2-Diphenyl picrylhydrazyl (DPPH), trichloroacetic acid, ferric chloride, potassium ferricyanide, sodium phosphate, ammonium molybdate, trypan blue. All chemicals used were of analytical grade.

Collection of plant material and extraction: The seeds were collected from the Coimbatore area in the year 2010. The plants & seeds were identified and authenticated by Prof. V.S. Ramachandran, Dept. of Botany, Bharathiar University, Coimbatore. The seeds of the plant *S. cumini* were thoroughly washed and dried at 37^{o}C. The dried seeds were further pulverized into fine powder. 25gm of the powdered seed was taken for the extraction purpose in ethanol as the solvent, by using Soxhlet's apparatus. This seed extract was used for studying the various antibacterial, *in vitro* antioxidant and cytotoxicity assays. All chemicals used were of analytical grade.

Phytochemical qualitative analyses: The aqueous and ethanolic extracts of the *S. cumini* seed were taken for carrying out various phytochemical analyses following the standard methods: Alkaloids with Mayer's test, flavonoids with alkaline reagent test, carbohydrates with Molisch test, glycosides with Legal's test, saponins using sodium bicarbonate, tannins using lead acetate, phytosterols with Salkowski's test, phenols using ferric chloride, triterpenoids with Libermann Buchard test, anthroquinones using concentrated sulfuric acid, benzene and ammonia and amino acids with Ninhydrin test. These were identified by characteristic color changes using standard procedures [21, 22].

GCMS analysis: The GCMS (Gas Chromatography Mass Spectroscopy) analysis of the *S. cumini* seed ethanolic extract was carried out at the South India Textile

Research Association (SITRA), Coimbatore. The Gas Chromatography (GC) was carried out by using Thermo GC Trace Ultra Version 5.0 equipment with run time of 35:32 mins and the Mass Spectrometry (MS) was carried out by using Thermo MS DSQ II equipment.

Antibacterial assay: The antibacterial activities of *S. cumini* seed ethanolic extract against different gram positive and gram negative bacteria were carried out by Agar well diffusion method on nutrient agar plates [23]. Test organisms used were *Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis and Pseudomonas aeruginosa.* Different concentrations (50 mg/ml- 250 mg/ml) of seed extract were made and the zone of inhibition was calculated for each concentration. Antibiotics such as Kanamycin (30mcg), Norphoxacin (10mcg) and Cephalothin (5mcg) were used as the standards. The Minimum inhibitory concentration (MIC) of the sample was determined by Serial tube dilution technique [24].

***In vitro* Antioxidant Assays:**

Determination of Total Phenolic Content: The total phenolic content of ethanolic extract of *S. cumini* seed was determined by Folin-Ciocalteau method [25,26]. The total phenolic content was expressed in terms of Gallic acid equivalent (mg/g of dry mass).

DPPH Free Radical Scavenging Assay: 1ml of 0.1mM 2, 2-Diphenyl picrylhydrazyl (DPPH) in ethanol, was added to different concentrations of *S. cumini* seed extract. The reaction mixture was well shaken and incubated in dark for 30 mins. Absorbance was checked at 517 nm against a blank (ethanol). Ascorbic acid was taken as the standard. Lower the absorbance of the reaction mixture indicates higher percentage of scavenging activity. The percentage of inhibition or scavenging of free radicals was determined by the formulae;

% Inhibition = [(Absorbance $_{Control}$ – Absorbance $_{Sample}$)**/** Absorbance $_{Control}$] x 100,

Where control was prepared as above without extract [27,28].

Reducing Power Assay: 500 µl of each concentration of seed extract was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (10g/l), then the mixture was incubated at 50º C for 20 minutes. 2.5 ml of trichloroacetic acid (100g/l) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1g/l) and absorbance measured at 700nm in UV-Visible Spectrophotometer. Ascorbic acid was used as standard and phosphate buffer used as blank solution. Increased absorbance of the reaction mixture indicates stronger reducing power [29, 30].

Total Antioxidant Capacity: 0.1 ml of different concentrations of seed extract were mixed in separate eppendorf with 1 ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate; mixed in 1:1:1 ratio) respectively. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After cooling to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Ascorbic acid was used as the standard and the total antioxidant capacity was expressed as equivalents of ascorbic acid [31].

Cytotoxicity Assay: The cytotoxicity activity of the seed ethanolic extract was done by Trypan blue dye exclusion technique [32]. The tumor cells were procured by using HeLa Cervical cancer cell line, purchased from National Centre for Cell Science, Pune. The tumor cells were maintained in RPMI-1640 medium. Different concentrations of extracts were prepared diluting with RPMI-1640 medium and were added to 100 µl of cell suspension. After 3 hours of incubation, percentage of mortality was calculated using trypan blue (0.4%) under haemocytometer, control was prepared same as above except extract. The percentage of mortality was calculated using the formulae: Percent (%) of mortality = (number of non viable cells/ total number of cells) x 100.

Statistical Analysis: All the grouped data were statistically evaluated with SPSS/16 software. Hypothesis testing methods included Student's t test followed by least significant difference (LSD) test. *P* values of less than 0.05 were considered to indicate statistical significance. Values are presented as the mean ± S.D. of each three replicates in each experiment.

RESULTS AND DISCUSSION:

Phytochemical Qualitative Analysis: A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index [33]. **Table 1** shows the presence of various phytoconstituents in the aqueous and ethanolic extracts of *S. cumini* seed. The performed qualitative studies indicate the presence of various phytoconstituents except glycosides and anthroquinones in the ethanolic extract of *S. cumini* seed. These phytoconstituents might be responsible for different biological activities and medicinal importance of this seed.

TABLE 1: THE PHYTOCHEMICAL CONSTITUENTS OF *S. CUMINI* SEED EXTRACTS (+ PRESENT, - ABSENT)

Phytochemical Constituents	Aqueous Extract	Ethanolic Extract
Alkaloids	+	+
Flavonoids	+	+
Carbohydrates	+	+
Glycosides	+	-
Saponins	+	+
Tannins	+	+
Phytosterols	+	+
Phenols	+	+
Triterpenoids	+	+
Anthroquinones	+	-
Amino acids	+	+

GCMS analysis of *S. cumini* seed ethanolic extract: **Figure 1** shows the GCMS chromatogram of the *S. cumini* seed ethanolic extract along with their retention time (RT). **Table 2** shows the major phytocomponents present in the *S. cumini* seed along with molecular formula, molecular weight, peak area and structure. The GCMS chromatogram of ethanolic extract of *S. cumini* seed showed the presence of compounds such as alkanes, sesquiterpenes, polyphenols.

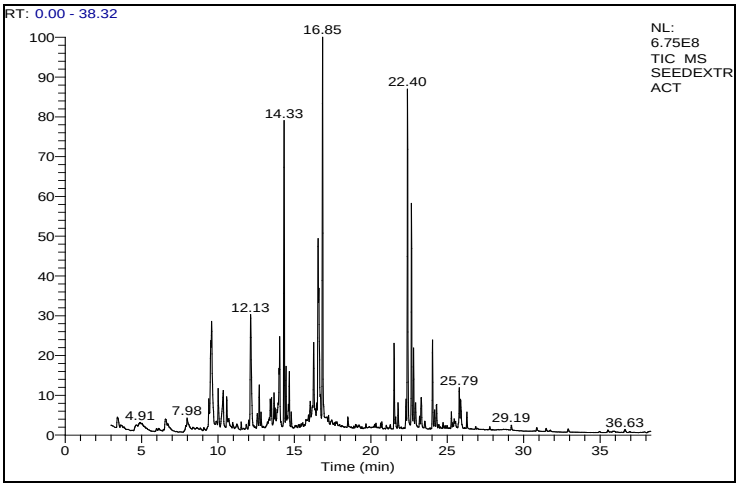
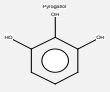
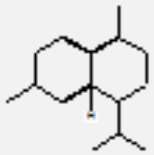
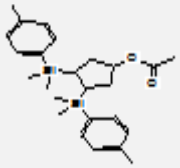
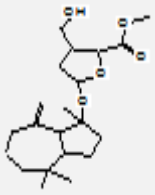


FIG. 1: THE GCMS CHROMATOGRAM OF *S. CUMINI* SEED ETHANOLIC EXTRACT

TABLE 2: GCMS ANALYTICAL REPORT FOR MAJOR PHYTOCONSTITUENTS IN ETHANOLIC EXTRACT OF *S. CUMINI* SEED

RT	Name of Compound	Molecular Formula	Molecular weight	Peak area	Structure
12.13	1, 2, 3-Benzenetriol (Pyrogallol)	$C_6H_6O_3$	126	7.31%	
14.33	ë-Cadinene (CAS)	$C_{15}H_{24}$	204	12.33%	
16.85	bicyclogermacrene	$C_{15}H_{24}$	204	13.66%	
22.40	(1a, 3a, 4a)-3, 4-Bis[dimethyl(4-methylphenyl)silyl]cyclopentan-1-yl acetate	$C_{25}H_{36}O_2Si_2$	424	11.23%	
25.79	shahamin B	$C_{21}H_{34}O_5$	366	2.01%	

Antibacterial Assay: The antibacterial activities of *S. cumini* seed ethanolic extract was studied against two gram positive bacteria such as *S. aureus* and *E. faecalis* and three gram negative bacteria such as *E. coli, K. pneumonia* and *P. aeruginosa* (**table 3**). The antibacterial activity of *S. cumini* seed extract was compared to the standard antibiotics (**table 4**). The seed extract showed to have potent antibacterial activity. The antibacterial effect was reported to be size and dose dependent.

TABLE 3: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *S. CUMINI* SEED

Microorganisms	50mg/ml	100mg/ml	150mg/ml	200mg/ml	250mg/ml
	Zone of inhibition in mm				
E. coli	10.3±0.57	12.3±0.58	14.3±0.57	16.3±1.53	18.0±1.00
S. aureus	8.7±0.58	11.7±0.58	14.3±1.53	16.7±1.52	19.7±1.53
P. aeruginosa	10.7±1.15	12.3±1.52	15.6±1.53	17.0±1.00	19.3±1.52
K. pneumoniae	10.0±2.00	11.6±1.53	14.7±1.15	18.6±1.52	21.7±1.15
E. faecalis	9.3±0.58	12.7±1.52	17.7±2.08	20.3±4.04	23.3±2.57

Values expressed as mean ± standard deviation and was calculated by the zone of inhibition

TABLE 4: ANTIBACTERIAL ACTIVITY OF STANDARD ANTIBIOTICS

Microorganisms	Kanamycin (30mcg)	Norfloxacin (10mcg)	Cephalothin (5mcg)
	Zone of inhibition in mm		
E. coli	10	14	18
S. aureus	8	10	12
P. aeruginosa	10	10	14
K. pneumoniae	12	16	18
E. faecalis	11	10	14

The minimum inhibitory concentration (MIC) represents the concentration of antimicrobial at which there is complete inhibition of growth of organism [34]. The MIC was determined by Serial tube dilution technique. The MIC value of the seed extract against *E. coli, P. aeruginosa* and *E. faecalis* was found to be 6.25mg/ml whereas against *S. aureus* and *K. pneumoniae*, MIC value was 3.13 mg/ml.

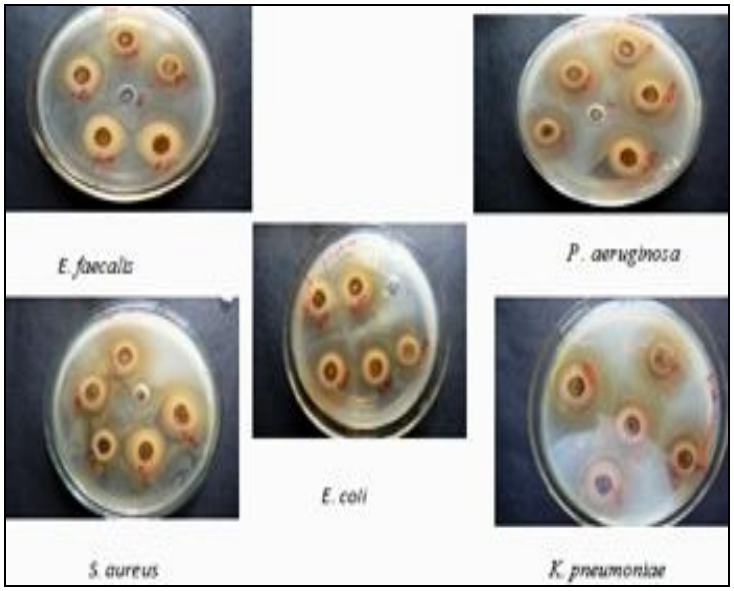


FIGURE 2: ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT OF *S. CUMINI* SEED EXTRACT

Total phenolic content: *S. cumini* is known to be very rich in gallic and ellagic acid polyphenol derivatives [35, 36]. The presence of high amount of polyphenols in the *S. cumini* seed was also evaluated from the estimation of total phenolic content of the seed extract by the Folin-Ciocalteau method, which was found to be 200.83±3.81 mg per gm of ethanolic extract of the *S. cumini* seed expressed as mg of Gallic acid equivalents. The phenolic compounds are very important plant constituents because of their antioxidant activities [37].

DPPH Free Radical Scavenging Assay: Figure 3 shows the DPPH Free Radical Scavenging Assay of *S. cumini* seed ethanolic extract (Sc) where ascorbic acid (AA) was taken as standard. A freshly prepared DPPH solution exhibited a deep purple color with a maximum absorption at 517 nm. This purple color disappears when an antioxidant is present in the medium. Thus, antioxidants molecules can quench DPPH free radicals and convert them to a colorless product, resulting in a decrease in absorbance at 517 nm [38]. The DPPH free radical scavenging assay showed potent inhibitory capacity of *S. cumini* seed extract when compared with ascorbic acid. The percentage of inhibition of free radicals increased with increase in concentration of substrates.

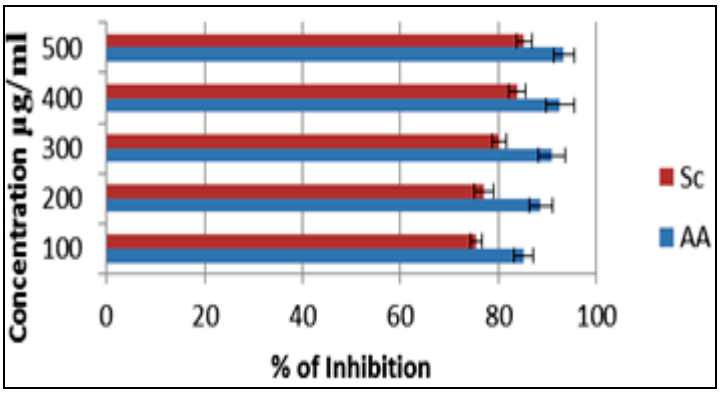


FIGURE 3: DPPH FREE RADICAL SCAVENGING ASSAY OF *S. CUMINI* SEED ETHANOLIC EXTRACT
(Values are mean ± SD of three determinations)

Reducing Power Assay: Figure 4 shows the Reducing Power Assay of *S. cumini* seed ethanolic extract (Sc) where ascorbic acid (AA) was taken as standard. The reducing ability of a compound depends on the presence of reductants [39] which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [40]. Presence of reducers causes the conversion of the Fe3+/ferricyanide complex used in this method to the ferrous form. By measuring the formation of Perl's Prussian blue at 700 nm, it is possible to determine the Fe2+ concentration [41]. *S. cumini* seed extract was found to have very high reducing ability when compared to the standard and increased with increasing concentration of substrates.

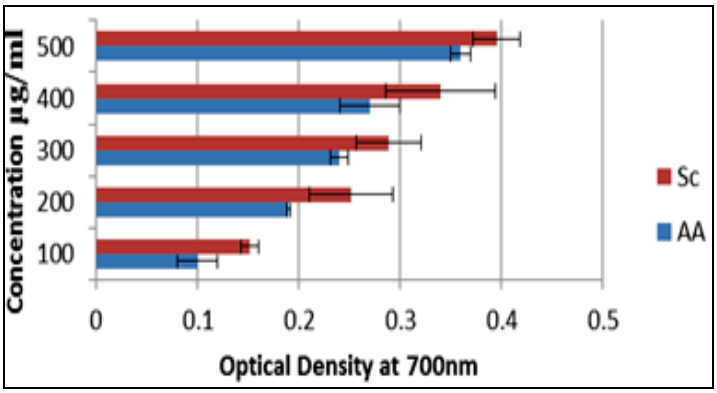


FIG. 4: REDUCING POWER ASSAY OF *S. CUMINI* SEED ETHANOLIC EXTRACT
(Values are mean ± SD of three determinations)

Total Antioxidant Capacity: Figure 5 shows the Total antioxidant capacity of ascorbic acid (AA) and *S. cumini* seed ethanolic extract (Sc). The Total antioxidant capacity was calculated based on the formation of the phosphomolybdenum complex where the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm [12]. The *S. cumini* seed extract was found to have very high total antioxidant capacity as compared to the standard.

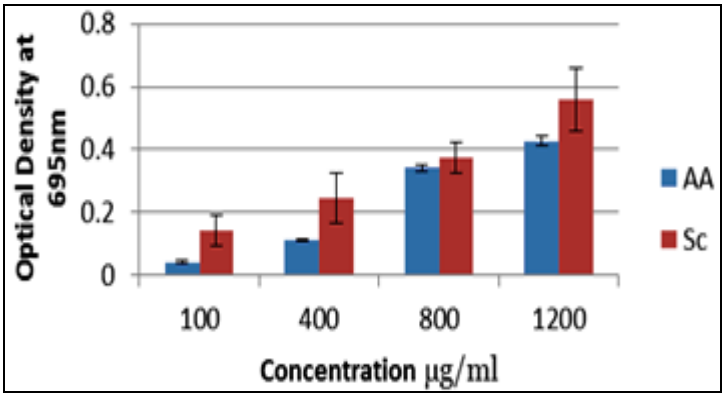


FIG. 5: TOTAL ANTIOXIDANT CAPACITY OF *S. CUMINI* SEED ETHANOLIC EXTRACT
(Values are mean ± SD of three determinations)

Cytotoxicity Assay: Figure 6 shows the growth inhibitory and cytotoxic activity of *S. cumini* seed ethanolic extract against cervical cancer cells. The viable cells remained unstained and appeared transparent and bright. The non- viable cells became stained and appeared blue in color. The growth inhibition and cytotoxic activity of *S. cumini* seed extract was carried out by Trypan blue dye exclusion assay. The growth inhibitory and cytotoxicity assay was found to be dose dependent. The extract showed significant decrease in cervical cancer cell population. The extract which causes at least 50% growth inhibition can be counted as cytotoxic [42]. The ethanolic extract of *S. cumini* seed, at a concentration of 0.5mg/ml showed more than 50% growth inhibition of cervical cancer cells.

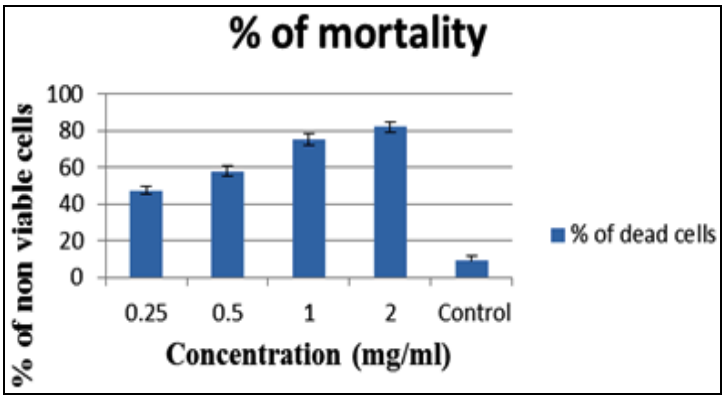


FIG. 6: CYTOTOXICITY ASSAY OF *S. CUMINI* SEED ETHANOLIC EXTRACT
(Values are mean ± SD of three determinations)

CONCLUSION: This study suggests that the ethanolic extract of *Syzygium cumini* seed has potent antibacterial, antioxidant and cytotoxic activities. Phytochemical studies showed the presence of polyphenols, tannins, flavonoids, terpenoids etc., which are responsible for the medicinal value of *S. cumini* seed. This seed extract has high antibacterial activity and thus can be used against multidrug resistant bacteria and as an herbal medicine alternative to the antibiotics. The seed extract of *S. cumini* contains high amount of polyphenolic components which may be responsible for its potential antioxidant activity.

Since, free radicals are important contributors to various degenerative diseases such as cancer. The observed antioxidant properties of the seed extract of *S. cumini* might be useful for the development of newer and more potent and natural antioxidant and thus can be used as potential free radical scavengers and can be used against the various damages caused by free radicals. The high growth inhibitory and cytotoxic effect of *S. cumini* seed extract against cervical cancer cells and thus can be used as potential anticarcinogenic agents. The *in vitro* bioassays provide an introspective knowledge of antibacterial, free radical scavenging and anticancerous activities of *S. cumini* seed and thus can be further investigated for *in vivo* studies.

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REFERENCES:

1. Dubey NK, Kumar R and Tripathi P: Global promotion of herbal medicine: India's opportunity. Current Science 2004; 86: 37-41.
2. Modi DC, Patel JK, Shah BN and Nayak BS: Pharmacognostic Studies of the Seed of *Syzygium cumini* Linn. Pharma Science Monitor 2010; 1: 20-26.
3. Parmar J, Sharma P, Verma P, Sharma P and Goyal PK: Chemopreventive Action of *Syzygium cumini* on DMBA - induced Skin Papillomagenesis in Mice. Asian Pacific Journal of Cancer Prevention 2010; 11: 261-265.
4. Gowri SS and Vasantha K: Free Radical Scavenging and Antioxidant Activity of Leaves from Agathi (*Sesbania grandiflora*) (L.) Pers. American-Eurasian Journal of Scientific Research 2010; 5: 114-119.
5. Prashanth KN, Neelam S, Chauhan S, Harishpadhi B and Ranjani M: Search for antibacterial and antifungal agents from selected Indian medicinal plants. Journal of Ethnopharmacology 2006; 107: 182-188.
6. Narendhirakannan RT, Subramanian S and Kandaswamy M: Free radical scavenging activity of *Cleome gynandra* L. leaves on adjuvant induced arthritis in rats. Molecular and Cellular Biochemistry 2005; 276: 71-80.
7. Wu D and Cederbaum AI: Alcohol, oxidative stress, and free radical damage. Alcohol Research and Health 2003; 27: 277-284.
8. Sen S, Chakraborty R, Sridhar C, Reddy YSR and De B: Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. International Journal of Pharmaceutical Sciences Review and Research 2010; 3: 91-100.
9. Kohen R and Nyska A: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicology and Pathology 2002; 30: 620-650.
10. Wichi HP: Enhanced tumor development by butylated hydroxyanisole (BHA) from the properties of effect on fure stomach and oesophagel aquamoua epithelium. Food Chemistry and Toxicology 1988; 26: 727-723.
11. Sherwin ER, Brancn AL, Davidson PM and Salmincn S: Food Additives. Marvel Dekker Inc, New York, 1990: 139-193.
12. Narendhirakannan RT and Smeera T: *In vitro* antioxidant studies on ethanolic extracts of leaves and stems of *Nyctanthes arbor-tristic* (L.) (Night-flowering Jasmine). International Journal of Biological and Medical Research 2010; 1: 188-192.
13. Elgorashi EE,Taylor JLS, Maes A,Van staden J, De Kimpe N and Verschaeve L: Screening of medicinal plants used in South African traditional medicine for genotoxic effects. Toxicology letters 2003; 143: 195-207.
14. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and McLaughlin JL: Brine shrimp: a convenient general bioassay for active plant constituents. Journal of Medicinal Plant Research 1982; 45: 31–34.
15. Kumar A, Padmanabhan N and Krishan MRV: Central Nervous System Activity of *Syzygium cumini* Seed. Pakistan Journal of Nutrition 2007; 6: 698-700.
16. Kirtikar KR and Basu BD: Indian Medicinal Plants. Periodical Experts, Vol. II, 1975: 1052-1053.
17. Nadkarni KM: Indian Materia medica. Popular book depot Bombay, Vol. I, 1954: 516-518.
18. Zhang LL and Lin YM: Antioxidant tannins from *Syzygium cumini* fruit. African Journal of Biotechnology 2009; 8: 2301-2309.
19. Ruan ZP, Zhang LL and Lin YM: Evaluation of the Antioxidant Activity of *Syzygium cumini* leaves. Molecules 2008; 13: 2545-2556.
20. Kumar A, Ilavarasan R, JayachandranT, Decaraman M, Kumar RM, Aravindhan P, Padmanabhan N and Krishan MRV: Anti-inflammatory activity of *Syzygium cumini* seed. African Journal of Biotechnology 2008; 7: 941-943.
21. Harborne JB: Phytochemical methods: A guide to modern techniques of plant analysis. Chapman and Hall, New York, Third Edition 1998.
22. Kokate CK: Pharmacohnosy. Nirali Prakasham, Mumbai, Sixteenth Edition 2001.

23. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing. Fifteenth Information Supplement M100-S15, Wayne, PA, USA, 2005.
24. Andrews JM: Determinations of minimum inhibitory concentrations. Journal of Antimicrobial Chemotherapy 2001; 48: 5-16.
25. Ebrahimzadeh MA, Pourmorad F and Bekhradnia AR: Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. African Journal of Biotechnology 2008; 32: 43-49.
26. Nabavi SM, Ebrahimzadeh MA, Nabavi SF, Hamidinia A, Bekhradnia AR. Determination of antioxidant activity, phenol and flavonoids content of *Parrotia persica Mey*. Pharmacologyonline 2008; 2: 560-567.
27. Tagashira M and Ohtake Y: A new antioxidative 1, 3-benzodioxole from *Melissa officinalis*. Planta Medica 1998; 64: 555-558.
28. Chang W, Choi CKS, Hwang SS, Bong KC, Hye JA, Min YL, Sang HP and Kim SK: Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay guided comparison. Plant Science 2002; 163: 1161-1168.
29. Koleva II, Van Beek TA, Linssen JPH, De Groot A and Evstatieva LN: Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochemical Analysis 2002; 13: 8-17.
30. Makari HK, Haraprasad N, Patil HS and Ravikumar: *In Vitro* Antioxidant Activity of the Hexane and Methanolic Extracts Of *Cordia Wallichii* and *Celastrus Paniculata*. Internet Journal of Aesthetic and Antiaging Medicine 2008; 1: 1-10.
31. Preito P, Pineda M and Aguilar M: Spectrophotometric quanification of antioxidant capacity through the formation of phosphomolybdnum complex: specific application of vitamin E. Anaytical Biochemistry 1999; 269: 337-341.
32. Shrivastava S and Ganesh N: Tumor inhibition and Cytotoxicity assay by aqueous extract of onion *(Allium cepa)* & Garlic *(Allium sativum)*: an *in-vitro* analysis. International Journal of Phytomedicine 2010; 2: 80-84.
33. Kumar A, Ilavarasan R, JayachandranT, Decaraman M, Aravindhan P, Padmanabhan N and Krishan MRV: Phytochemicals Investigation on a Tropical Plant, *Syzygium cumini* from Kattuppalayam, Erode District, Tamil Nadu, South India. Pakistan Journal of Nutrition 2009; 8: 83-85.
34. Islam MA, Alam MM, Choudhury ME, Kobayashi N and Ahmed MU: Determination of minimum inhibitory concentration (mic) of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (mrsa) with their antibiogram. Bangladesh Journal of Veterinary Medicine 2008; 6: 121–126.
35. Chattopadhyay D, Sinha BK and Vaid LK: Antibacterial activity of *Syzygium* species. Fitoterapia 1998; 69: 356-367.
36. Chung KT, Wei C and Johnson MG: Are tannins a double edged sword in biology and health. Trends in Food Science and Technology 1998; 9: 168-175.
37. Paganga G, Miller N and Rice-Evans CA: The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radical Research 1999; 30: 153-162.
38. Miladi S and Damak M: *In vitro* antioxidant activities of *Aloe vera* leaf skin extracts. Journal de la Société Chimique de Tunisie 2008; 10: 101-109.
39. Duh PD, Tu YY and Yen GC: Antioxidant activity of water extract of *Harng Jyur (Chrysanthemum moifolium* Ramat). Lebensmittel–Wissenschaft und Technologie 1999; 32: 269-277.
40. Schimada VL, Fujikawa K, Yahara K and Nakamura T: Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 1992; 40: 945-948.
41. Narendhirakannan RT and Limmy TP: *In vitro* antioxidant studies on ethanolic extracts of leaf, stem and root of *Sida rhombifolia* (L.). International Journal of Pharma and Biosciences 2010; 1: 1-10.
42. Sadeghi-Aliabadi H, Sajjadi SE and Khodamoradi M: Cytotoxicity of *Euphorbia macroclada* on MDA-MB-468 Breast Cancer Cell Line. Iranian Journal of Pharmaceutical Sciences 2009; 5: 103-108.

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