An investigation of the *Syzygium cumini* L. seed extract against ovarian cancer: *In vitro studies*

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Abstract

Medicinal plants nurture various chemical constituents that can be extracted and used for various ailments like anticancer, antibacterial, anti-diabetic, anti-inflammatory and antioxidants. In this pipeline, we explore the anticancer activity of Syzygium cumini L. seed extracts against ovarian cancer. Our study shows that Syzygium cumini L. seed extract triggers apoptosis-mediated cell death in the SKOV cell line. Further, we uncovered that Syzygium cumini L. seed extract triggers ROS and induces oxidative stress followed by apoptosis. The cell cycle distribution analysis by flow cytometry denotes that Syzygium cumini L. seed extract arrests the cell cycle at the Go/G1 phase. The GC-MS analysis of the Syzygium cumini L. seed acetone extract implies the presence of notorious bioactive compounds claiming to have antioxidant, antibacterial, antifungal and antioxidant, anticancer and anti-inflammatory properties. Thus, in the overall study we conclude that Syzygium cumini L. seed extract can be developed as a lead molecule for the treatment of ovarian cancer after subjecting to in vivo studies.

Keywords: *Syzygium cumini*, seed, ovarian cancer, antioxidant, acetone, SKOV cell line.

Introduction

Ovarian cancer is the most predominant cancer among women affecting the ovaries, fallopian tubes and the primary peritoneal cavity[18]. The most common form of ovarian cancer is epithelial ovarian cancer which also includes fallopian tube cancer and primary peritoneal cancer[16]. Ovarian cancer causes severe morbidity and mortality rates in women in various developing countries with an estimated 2, 39, 000 cases and 152000 death occurring annually[20]. The cancer proliferates at the age of >75 and survives for 5 years.

Generically, ovarian tumors originate from epithelial, germ cells, stromal cells. Among ovarian cancer, 96% of ovarian cancers originate from epithelial followed by stromal and germ cells[12]. Histological subtypes exist in ovarian cancer that differ in prognosis, cellular origin, molecular alterations and gene expressions. The causative factors for ovarian cancer are estrogen hormone replacement therapy, tobacco, smoking, alcoholism, physical inactivity, lifestyle and obesity[19]. The current treatment regime for the treatment of ovarian cancer includes surgery and non-surgery methods. Drugs like paclitaxel, cisplatin, carboplatin, doxorubicin bevacizumab, 5-fluorouracil, leucovorin, oxalplatin are used[23]. The treatment reflects heavy adverse side effects to the patients and sometimes acquires resistance against the treatments by the cancer cells. Henceforth, there is need to discover new drug sources to combat the clinical manifestations in cancer treatment.

Historically, the use of plants and their products has been practiced in Ayurvedic medicine against infectious and non-infectious diseases. Plants-based therapy offers non-toxicity, prolonged effects and lower side effects. Over the last few decades, various studies based on plant extracts strongly annotate the anticancer effects[1]. In some studies, plant-based entities (vinblastine, vincristine, topotecan irinotecan, etoposide, paclitaxel roscovitine, betulinic acid and silvestrol) are considered as lead molecules for the drug development in cancer chemotherapy[24].

Syzygium cumini L. is a medicinal herb known as Jamun and is widespread all over the Indian subcontinent. Traditionally, fruits and seeds were widely used for the treatment of various metabolic disorders and infectious and noninfectious diseases. As per the literature, *Syzygium cumini* seed possesses astringent and diuretic, hypoglycaemic, anti-inflammatory, antipyretic, psychopharmacological, hypolipidaemic and antioxidant activities[14].

In the present study, we intend to study the anti-cancer activities of *S. cumini* seed extract against ovarian cancer. This is accomplished by the solvent extraction of seeds and its subsequent activity in the ovarian cancer cell line.

Material and Methods

Materials: *Syzygium cumini* seed, solvents, chemicals, cell lines and others utilized in the study were commercially purchased from leading scientific companies.

Sample Preparation and Collection: The *Syzygium cumini* fruit was obtained from the agricultural fields adjacent to Western Ghats of Coimbatore, India (Fig. 1). The freshly collected fruits were brought immediately to the laboratory and washed. After the washing process, the peels of the fruits were removed and the seeds were obtained and washed immediately. The obtained seeds were dried in a hot air oven at 40°C for 3 days. After drying, the dried seeds were macerated using a blender for fine powder formation. The obtained powder was used for further studies. To prepare,

Syzygium cumini seed extract 100 gm of powder was weighed and poured individually into a bottle filled with respective solvents (methanol, ethanol, acetone, hexane and chloroform).

The mixture bottles were kept in an orbital shaker at 1000 rpm for 3 days. After the incubation period, the mixtures were filtered through the Whatmann filter paper and the extract was subjected to a rotary vacuum evaporator to remove the solvents from the mixture. The obtained crude extract was used for further studies.

Cytotoxicity study: SKOV (Human ovarian cancer) cell line was initially procured from the National Centre for Cell Sciences (NCCS), Pune, India. To the Dulbecco's modified Eagle's medium, DMEM, 10% FBS, L-glutamine, sodium bicarbonate penicillin (100U/ml), streptomycin (100µg/ml) and amphotericin B (2.5µg/ml) were added and kept at 37ºC in a humidified 5% CO_2 incubator. The two days monolayer cells were cultured and trypsinized and the cells were suspended in the medium. From the medium, 100 cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator. For the cytotoxicity study, 1mg of seed extracts was weighed and dissolved in 1 ml 0.1% DMSO using a cyclomixer.

The sample solution was filtered through a 0.22 µm Millipore syringe filter to ensure sterility. The samples were added to the medium at concentrations of 100µg, 50µg, 25µg, 12.5µg and 6.25µg in 500 of DMSO and each concentration of 100 was added in triplicate to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator[6].

After 24 hours of incubation period, the extract mixture in the wells was removed and 30µl of MTT solution was added to all the wells and incubated at 37ºC in a humidified 5% CO_2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilization solution (Dimethyl sulphoxide, DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down to solubilize the formazan crystals. The absorbance values were measured by using a microplate reader at a wavelength of 540 nm[21].

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples x } 100}{\text{Mean OD of the control group}}$$

Fig. 1: Seeds of the fruit *Syzygium cumini* L.

AO/EB staining: The AO/EB staining was performed to ascertain whether the *Syzygium cumini* seed extract triggered apoptosis in the ovarian cancer cell line[21]. For analysis, based on the results of the cytotoxicity assay, we chose the acetone extract for further studies. The *Syzygium cumini* seed acetone extract IC_{50} 79.47683µg/mL from a stock of 1mg/ml was added and incubated for 24 hours. Non-treated controls were also maintained and incubated for 24 hours. After incubation, the cells were washed with cold PBS and then stained with a mixture of AO (100 µg/ml) and EtBr (100 µg/ml) at room temperature for 10min. The stained cells were washed twice with 1X PBS and observed by a fluorescence microscope in a blue filter of a fluorescent microscope (Olympus CKX41 with Optika Pro5 camera)

ROS assay: The SKOV cell line was cultured in the respective medium and treated with *Syzygium cumini* seed acetone extract IC_{50} 79.47µg/mL. 1mg/ml was added and incubated for 24 hours. After the incubation period, the cells were washed with PBS. Add 50 µl of DCFDA and incubate for 30 minutes[6]. After incubation, excess dye was washed with PBS and fluorescence was imaged in a fluorescent microscope (Olympus CKX41 with Optika pro5 CCD camera) and fluorescence was measured using a fluorimeter at 470 nm excitation and emission at 635nm (Qubit 3.0, Life Technologies, USA) and expressed in arbitrary units.

Lactate Dehydrogenase Assay (LDH): The SKOV cell line was cultured in the respective medium and treated with *Syzygium cumini* seed acetone extract IC_{50} 79.47 µg/mL. 1mg/ml was added and incubated for 24 hours. After the incubation period, the LDH assay was performed according to the manufacturer's kit protocol (Calkine LDH kit –IFCC Method).

Flow cytometry analysis: The flow cytometry analysis was performed to annotate the cell's ability to proliferate during toxicity studies[9]. The most accurate method of doing this is by directly measuring DNA synthesis. The analysis was performed according to the manufacturer kit protocol MUSE cell cycle kit which employs the nuclear DNA intercalating stains propidium iodide (PI) which discriminates cells at different stages of the cell cycle based on the differential DNA content in the presence of RNAase to increase the specificity of DNA staining in each phase (G_0/G_1, S and G_2/M).

GC-MS analysis: GC-MS analysis was performed in the acetone extract to identify the compounds present in the extract. For analysis, the GC-MS QP 2010 (Shimadzu) instrument was used and the results were compared by using the National Institute of Standards and Technology (NIST) Spectral Library search program.

Results and Discussion

Over the last three decades, numerous studies have been carried out on various plant extracts against various cancers. The results of the studies are promising and some results have developed a prototype for cancer treatments. The lead compounds developed from plants showed unique distinctive properties like synthetic drug molecules[7]. In addition, the natural compounds have a high degree of structural complexity with a high molecular mass, high number of oxygen and carbon atoms, high number of H-bond acceptors and donors and a lower (cLogP values) and high rigidity[26]. These properties of natural compounds tackle protein–protein interactions in the drug discovery process.

Cytotoxicity study: The cytotoxicity study is a preliminary clue to explore the anti-therapeutic properties of the extracts. In the present study, we have evaluated the *Syzygium cumini* seed extracts (Acetone, Methanol, Chloroform, Ethanol and Ethyl acetate) in the ovarian cancer cell line SKOV. As a result, the solvent extracts performed superior activity in the cell lines and inhibited the growth of the cells in a dose-dependent manner (Fig. 2). The IC_{50} values for the solvent extract were significant and the acetone solvent extract had the lowest IC_{50} value 79 µg/mL when compared with other solvent extracts.

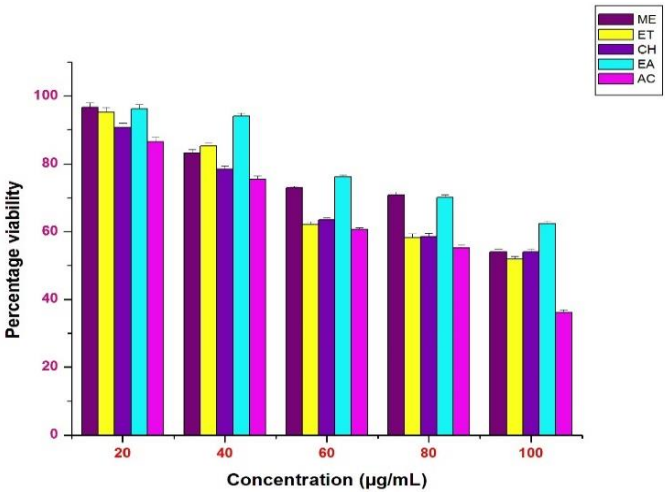


Fig. 2: Cytotoxic assay of *S.cumini* seed extracts against SKOV cell line. The results are the mean value of three replicates and statistically analyzed

In the present study, we have observed that acetone extract of *S.cumini* seed performed strong inhibitory activity against the SKOV cell line. This is due to the active constituents trapped in the acetone extract. Table 1 provides the anticancer activity of *S.cumini* seed extract performed in various cancer cell lines. The table infers that *S.cumini seed* extract activity differs with respect to the cell lines and seed constituents. In the present study, our *S.cumini* seed extract (acetone) performed better in the SKOV cell line. Acetone is having low polarity index.

In addition, the geographical location will also influence the phytoconstituents in the plants[3]. We sampled the *S.cumini* seed from the Western Ghats of Coimbatore (11.0168° N, 76.9558° E), India and thus there will be variable phytomolecules in the seed. Henceforth, in the present study, the *S.cumini* seed extract was extracted effectively by the acetone solvent for enhanced anticancer activity while compared with other solvent extracts.

The acetone extract causes lethal mortality in the cell lines. It is obvious that according to the dose concentrations, the acetone triggers the activity. The extract constituents interrogate the cell functions by inducing apoptosis and cell death. Fig. 3 indicates apoptotic cell bodies with cell membrane disruption, the flow of internal cytosolic organelles and irregular aggregates. Further, molecular studies were performed with the IC_{50} µg/mL value of the acetone extract.

AO/EB staining: To further interrogate that *S.cumini* seed acetone extract induces apoptosis in the cell line SKOV, we assayed the AO/EB staining to authenticate the apoptosis (Fig. 4). The acetone extract causes the cells to undergo apoptosis and as a result, there will be a change in the cell morphology such as cell membrane damage, blebbing, cell shrinkage, nuclear fragmentation and condensation[27]. Green-colored fluorescence indicates the viable cells, while orange/red color fluorescence denotes the proapoptotic and apoptotic cells. Thus from the microscopy image, it is evident that acetone extract causes apoptosis in the cells and causes cell death.

ROS assay: In the present study, *S.cumini* seed acetone extract IC_{50} value 79 µg/mL promotes ROS generation significantly in the cell line. In the fig. 5, the control cells did not observe any green fluorescence indicating no production of ROS; while the treated cells emitted green fluorescence indicating the production of ROS upon the treatment of *S.cumini* seed acetone extract. ROS accumulation in the cells and failure of the antioxidant defense system cause the cells to undergo apoptosis[11]. Generally, higher mitochondrial membrane potential causes a significant increase in Adenosine triphosphate production (ATP) and thereby increases ROS[15]. These intracellular ROS were measured using the intensity of highly fluorescent derivative 2′,7′-dichlorofluorescein (DCF) generated from a non-fluorescent substrate DCFH-DA.

LDH assay: Measurement of LDH is a qualitative method to assess the cytotoxicity of the drugs. Intracellular LDH is released into the medium when the cell membrane integrity is damaged due to the toxic effects[17]. In *S.cumini* seed acetone extract (0 -100 µg/mL), there was a significant increase in the LDH as depicted in fig. 6. At high concentrations, there was more amount of enzyme released from the cells. Thus, it can be inferred that *S.cumini* seed acetone extract exerts ROS increase and damages the internal cell organelles which eventually releases LDH into the medium. Hence it is evident that *S.cumini* seed acetone extract causes apoptosis in the ovarian cancer cell line.

Flow cytometry analysis: Phytomolecules proved to be cell cycle modulators due to the process of apoptosis and of cell cycle inhibition. Herein the present study, we have analyzed the checkpoints of the cell cycle using flow cytometry. The analysis was performed to evaluate the effect of DNA content in response to the treatment of *S.cumini* seed acetone extract (IC_{50} value 79 µg/mL) in the SKOV cell line. In fig. 7, it is indicated that *S.cumini* seed acetone extract (IC_{50} value 79 µg/mL) significantly increased the accumulation of the DNA contents up to 79.1% in the G0/G1 phase while in the S and G2/M phase, there was a drop out in the DNA content up to 10.3% and 5.2% respectively while compared with control[2]. From the fig. 7, it is evident that *S.cumini* seed acetone extract arrests the cell in the G0/G1 phase of the cell cycle.

Table 1
Anti-cancer activiry of *S.cumini* seed extract

S.N.	*Syzygium cumini L.* Seeds	Cell Line	IC_{50} µg/mL
1.	Ethanolic extract[8]	A2780 MCF7 PC-3 H460	49 110 140 165
2.	Methanolic Extract[15]	HepG2 Caco2 Pc3	89 39 38
3.	Hexane Ethanol[10]	MDA-MB-231	195 142

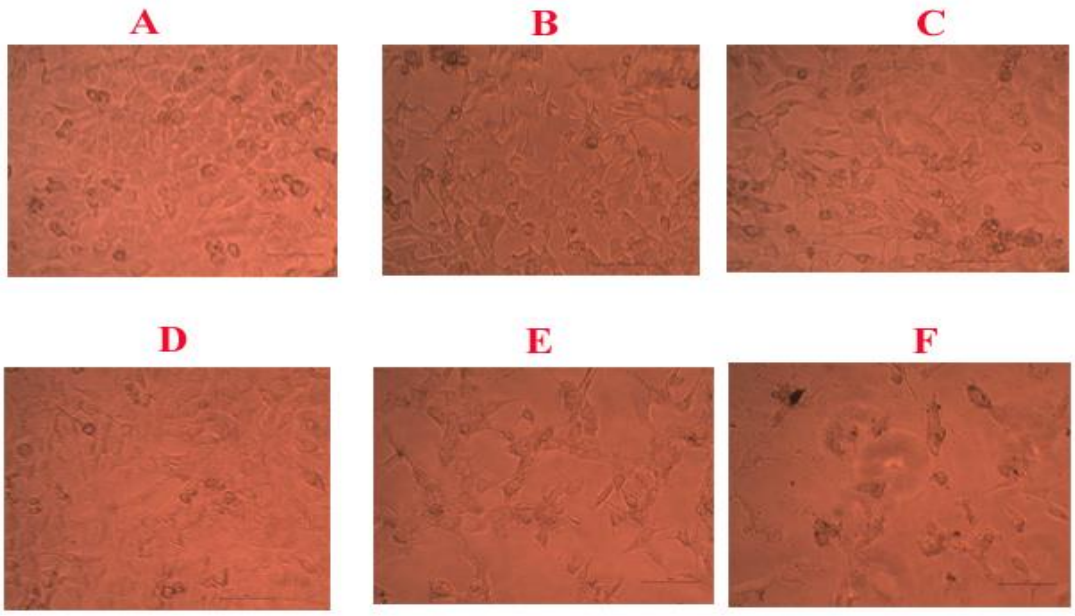


Fig. 3: Microscopic examination of SKOV cell line treated with acetone extract.

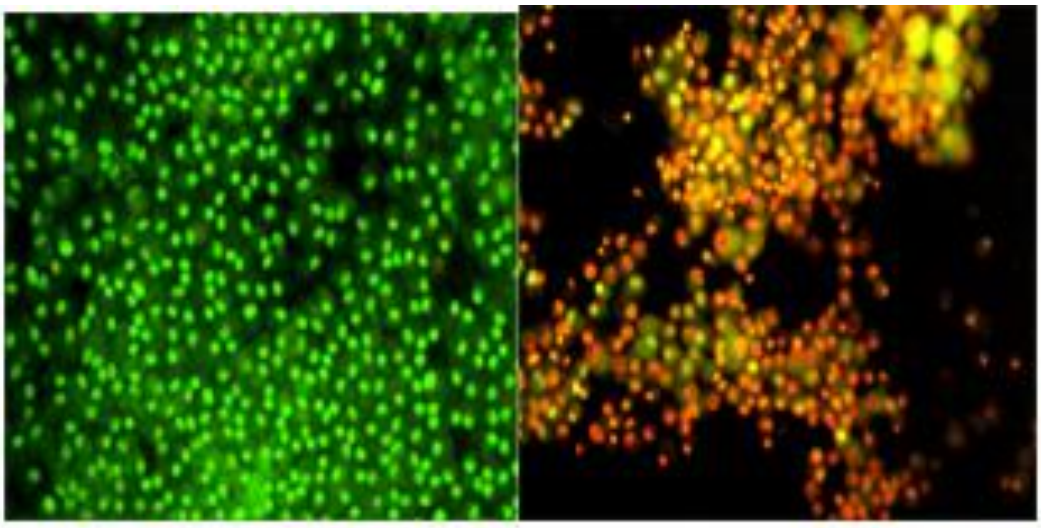


Fig. 4: AO/EB staining assay of *S.cumini* seed acetone extract.

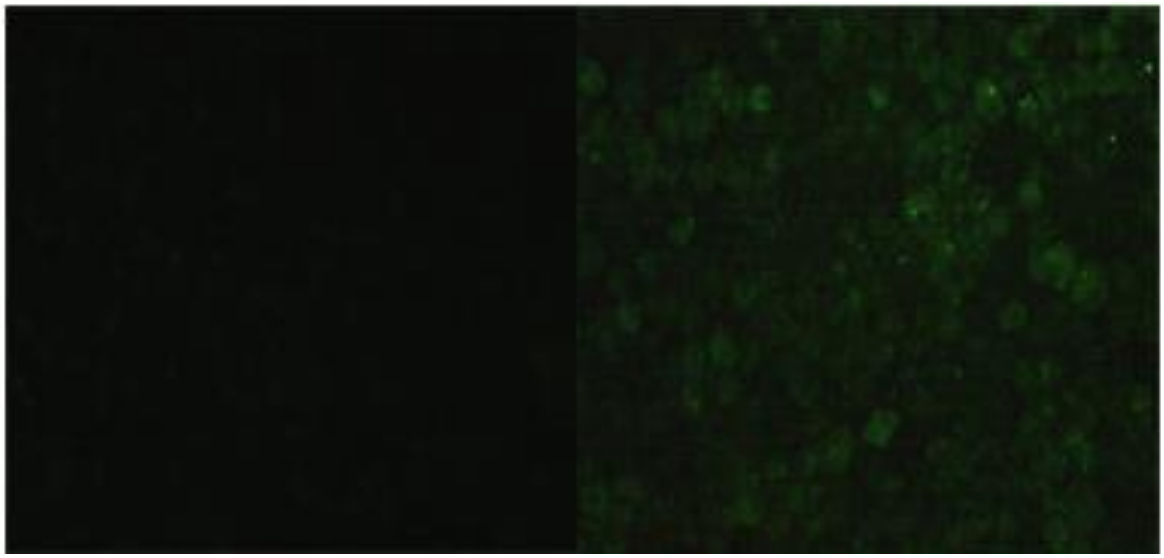


Fig. 5: ROS assay of *S.cumini* seed acetone extract in SKOV cell line.

Table 2
Identification of the compounds from the acetone extract of *S.cumini*

S.N.	Peak Number	Compounds	Retention time	Percentage
1	1	1,3-Dioxolane-4-methanol, 2,2-di...	4.320 min	6.47%
2	2	2-Hexanol, 2,3-dimethyl	4.375 min	0.13%
3	3	Butanamide, 3-methyl-	4.564 min	0.59%
4	4	Thiophene, 2-ethyltetrahydro-	4.886 min	0.14%
5	5	Benzene, 1-methyl-2-(1-methyleth...	5.086 min	0.28%
6	6	3-Allyloxy-1,2 propanediol	5.320 min	0.09%
7	7	Benzene, 1-methyl-4-(1-methyleth...	5.775 min	0.25%
8	8	(E)-3(10)-Caren-4-ol	6.353 min	0.23%
9	9	4H-Pyran-4-one, 2,3-dihydro-3,5-...	6.43 min	0.18%
10	10	4,4'-Bi-1,3-dioxolane, 2,2,2',2'...	6.531 min	0.98%
11	11	Di-sec-butyl ether	6.897 min	0.75%
12	12	Benzofuran, 2,3-dihydro-	7.064 min	1.08%
13	13	2-Furancarboxaldehyde, 5-(hydrox...	7.308 min	0.94%
14	14	Resorcinol	7.886 min	2.78%
15	15	Phthalic anhydride	8.064 min	0.35%
16	16	3-Pyridinol, 6-methyl-	8.175 min	0.73%
17	17	Eugenol	8.353 min	0.18%
18	18	Dimethylallyl(n-octyl)silane	8.519 min	0.16%
19	19	Copaene	8.597 min	0.30%
20	20	1,2,3-Benzenetriol	8.775 min	4.98%
21	21	Isoledene	9.197 min	0.28%
22	22	alpha.-Caryophyllene	9.341 min	0.65%
23	23	Aromadendrene	9.375 min	0.33%
24	24	Longifolene-(V4)	9.453 min	1.60%
25	25	Naphthalene, 1,2,4a,5,6,8a-hexah...	9.664 min	2.80 %
26	26	1H-Cycloprop[e]azulene, 1a,2,3,4...	9.719 min	0.94%
27	27	Naphthalene, 1,2,3,5,6,8a-hexahy...	9.830 min	3.52%
28	28	Naphthalene, 1,2,3,4-tetrahydro-...	9.875 min	0.78%
29	29	Naphthalene, 1,2,4a,5,6,8a-hexah...	9.986 min	0.74%
30	30	alpha.-Calacorene	10.041 min	0.74%
31	31	1,6,10-Dodecatrien-3-ol, 3,7,11-...	10.097 min	0.38%
32	32	6H-Cyclopenta[e][1,2,4]triazolo[...	10.208 min	0.15%
33	33	2-Phenyl-2,4-octadienol	10.308 min	0.53%
34	34	1H-Cycloprop[e]azulen-7-ol, deca...	10.364 min	0.68%
35	35	Azulene, 1,2,3,3a,4,5,6,7-octahy...	10.464 min	0.47%
36	36	Benzene, 1,2,4-trimethoxy-5-(1-p...	10.497 min	0.43%
37	37	Naphthalene, 1,2,3,4,4a,5,6,8a-o...	10.641 min	0.61%
38	38	1H-Cycloprop[e]azulen-7-ol, deca...	10.763 min	1.02%
39	39	Bicyclo[4.4.0]dec-1-ene, 2-isopr...	10.863 min	2.85%
40	40	Epiglobulol	10.986 min	3.72%
41	41	.alpha.-Bisabolol	11.141 min	1.12%
42	42	cis-Z-.alpha.-Bisabolene epoxide	11.208 min	0.32%
43	43	2(1H)-Naphthalenone, 4a,5,6,7,8,...	11.275 min	0.28%
44	44	3-Bromo-7-methyl-1-adamantanecar...	11.363 min	0.50%
45	45	Neoisolongifolene, 8,9-dehydro-	11.430 min	0.28%
46	46	Cyclohexane, 1,5-diethenyl-3-met...	11.508 min	0.47%
47	47	Tetradecanoic acid	11.619 min	1.12%
48	48	1H-3a,7-Methanoazulene, octahydr	11.774 min	0.17%
49	49	Azulene, 1,4-dimethyl-7-(1-methy...	11.863 min	0.76%
50	50	4-Methyl-2,6-dihydroxyquinoline	12.008 min	0.21%
51	51	Bicyclo[3.1.1]heptane, 2,6,6-tri...	12.119 min	0.29%
52	52	Longifolenaldehyde	12.152 min	0.17%

53	53	trans-Z-.alpha.-Bisabolene epoxide	12.197 min	0.12%
54	54	trans-.alpha.-Bergamotene	12.319 min	1.47%
55	55	2,4-Methano-2H-indeno[1,2-b:5,6-...	12.419 min	0.21%
56	56	Caryophyllene oxide	12.530 min	0.25%
57	57	Pent-1-yne, 5-benzyloxy-	12.608 min	0.48%
58	58	Pentadecanoic acid, 14-methyl-, ...	12.730 min	0.54%
59	59	.alpha.-Farnesene	12.785 min	0.31%
60	60	1 Germacra-1(10),4,11(13)-trien-12...	12.830 min	0.12%
61	61	9-Hexadecenoic acid	12.908 min	0.32%
62	62	n-Hexadecanoic acid	13.208 min	15.73%
63	63	Tridecanoic acid	13.330 min	0.11%
64	64	n-Hexadecanoic acid	13.396 min	0.09%
65	65	n-Hexadecanoic acid	13.452 min	0.12%
66	66	13-Octadecenal, (Z)-	13.563 min	0.20%
67	67	Tridecanoic acid	13.674 min	0.20%
68	68	9,12-Octadecadienoic acid, methy...	13.841 min	0.13%
69	69	9-Octadecenoic acid (Z)-, methyl...	13.874 min	0.17%
70	70	9,12-Octadecadienoic acid (Z,Z)-	14.319 min	22.42%
71	71	Octadecanoic acid	14.452 min	3.01%
72	72	Eicosanoic acid	15.463 min	0.31%
73	73	Hexadecanoic acid, (2,2-dimethyl...	16.029 min	0.18%
74	74	Tricosane	16.141 min	0.12%
75	75	1,2-Benzenedicarboxylic acid, mo...	16.352 min	0.11%
76	76	Benzamide, 2-amino-N-(4-methoxyp...	16.396 min	0.17%
77	77	Hexacosane	16.663 min	0.18%
78	78	2-Isobutoxy-4-methyl-[1,3,2]diox...	16.940 min	0.24%
79	79	1-Ethyl-1-cyclopentyloxy-1-silac...	17.052 min	0.09%
80	80	Hexadecane	17.163 min	0.46%
81	81	Hexadecane	17.685 min	0.12%
82	82	Squalene	17.796 min	0.11%
83	83	Heptadecane	18.285 min	0.21%
84	84	Vitamin E	20.107 min	0.12%
85	85	Campesterol	22.318 min	1.07%

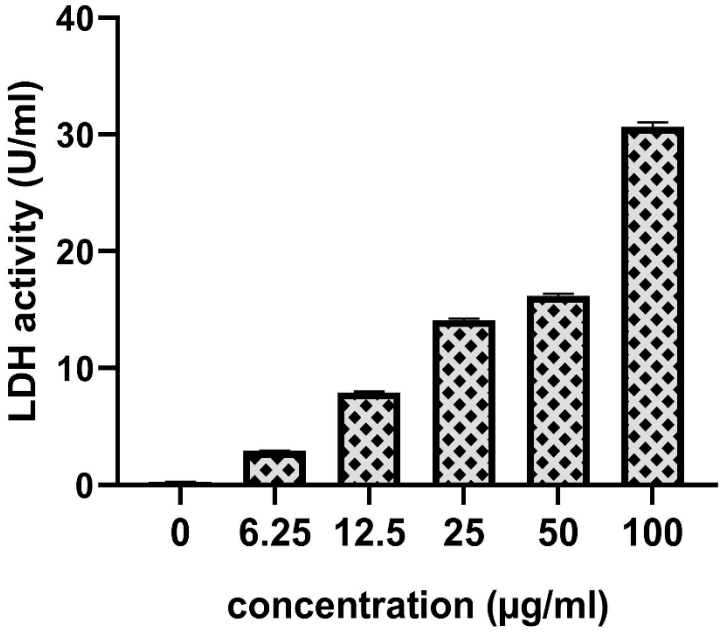


Fig. 6: LDH assay of *S.cumini* seed acetone extract in SKOV cell line.

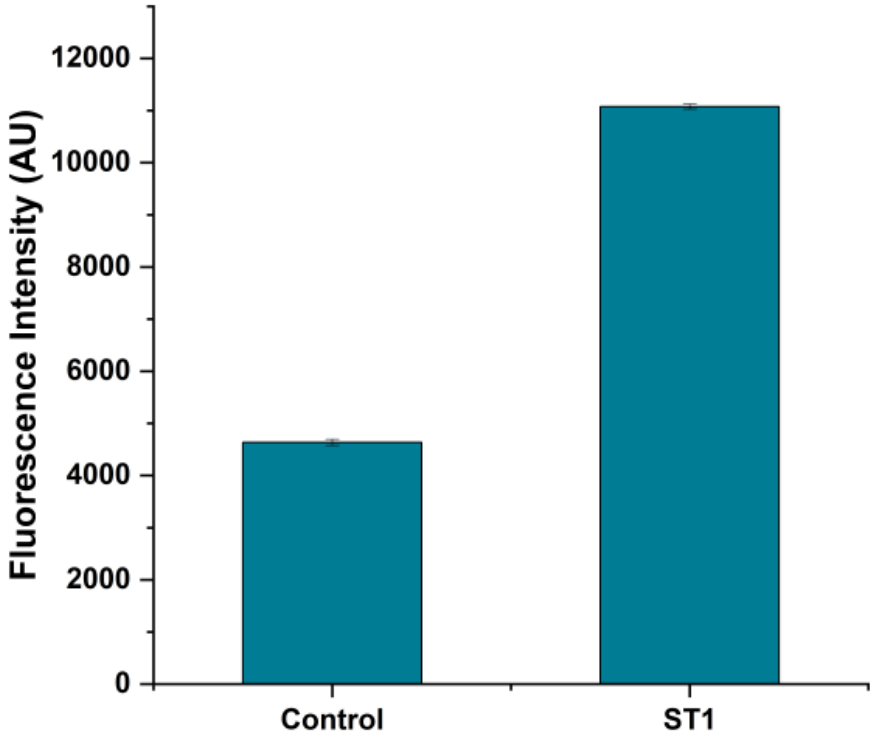
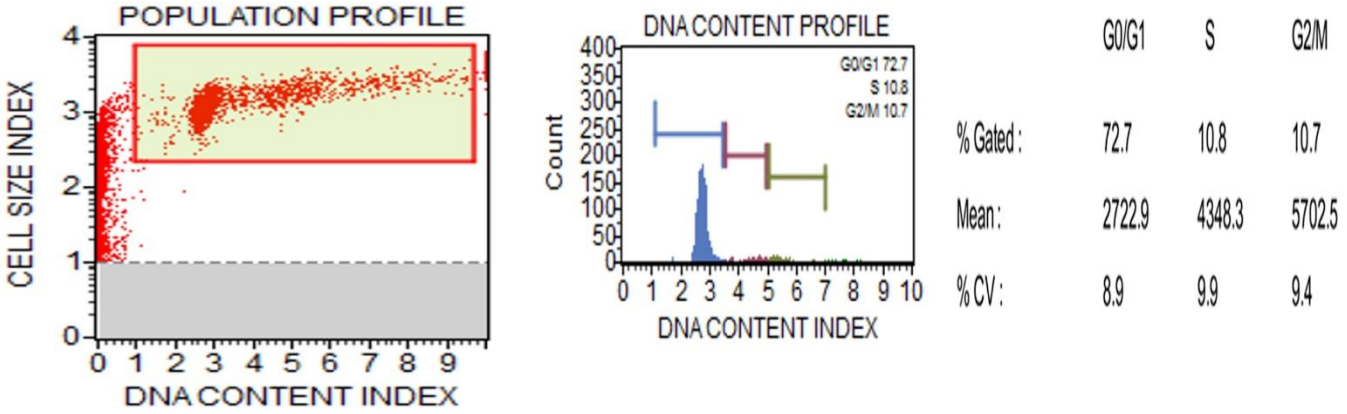
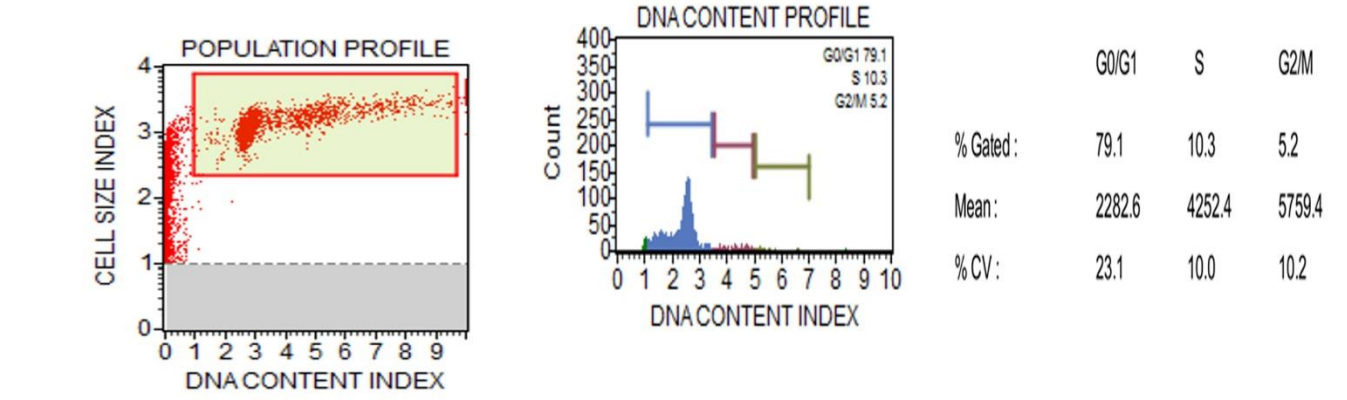


Fig. 7: Flow cytometer analysis of *S.cumini* seed acetone extract in SKOV cell line





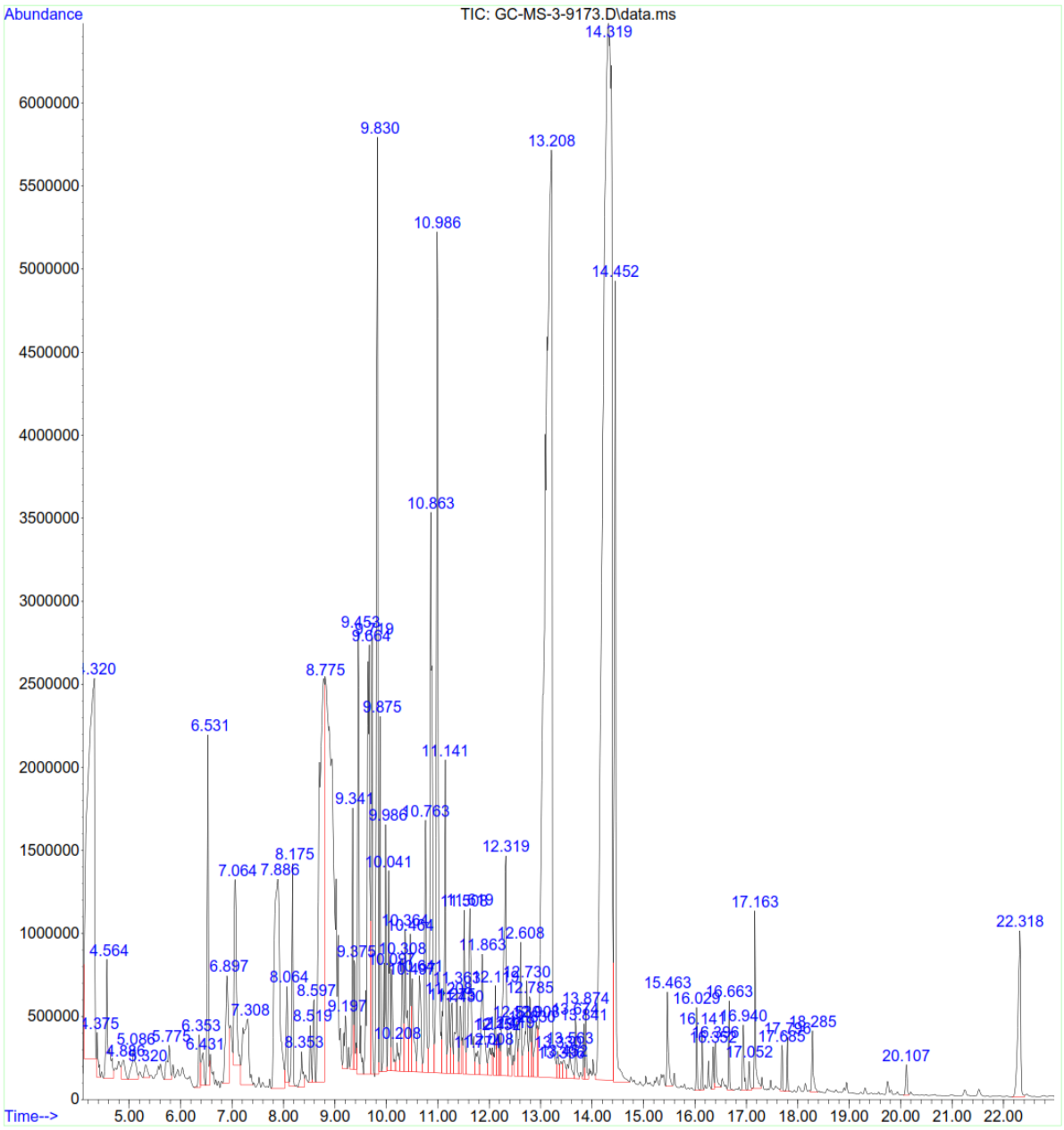


Fig. 8: GC-MS spectrum of *S.cumini* seed acetone extract

This phenomenon of cell cycle arrest is attributed to the early apoptosis triggered by the *S.cumini* seed acetone extract. Reports of the extract of *Tinospora cordifolia* and *Marsdenia tenacissima* also have similar effects arresting the cell cycle at the G0/G1 phase[22].

GC-MS analysis: The acetone extracts were performed to ascertain the active phyto compounds present in the extract (Fig.8). The spectrum and mass of the compounds were recorded and identified with the NIST database. As a result, a total of 86 peaks with their corresponding compounds, retention time and other details were represented in table 1.

From the table, we peculiarly noted that Resorcinol, Copaene, Isoledene, Epiglobulol, Tetradecanoic acid, Longifolenaldehyde Caryophyllene oxide, Pentadecanoic acid, 9-hexadecenoic acid, Octadecanoic acid, Eicosanoic acid, Squalene Heptadecane, Vitamin E and Campesterol, the identified compounds in the GC-MS analysis are known to attribute the various therapeutic properties (antioxidant, antibacterial, antifungal, antioxidant, anticancer and anti-inflammatory properties) as reported in literature[5,22,25].

Conclusion

The present strongly interrogates the anticancer activities of the *S.cumini* seed extracts. The *S.cumini* seed acetone extract displayed prominent activity in the preliminary cytotoxicity study in the SKOV cell line. Following the MTT assay, the *S.cumini* seed acetone extract triggered apoptosis as observed by the AO/EB staining. Further, the acetone extract induces the generation of ROS and increase of LDH ssacetone induces oxidative stress-mediated apoptosis.

In addition, *S.cumini* seed acetone extract also inhibits the cell cycle at the GO/G1 phase. The GC-MS analysis infers that *S.cumini* seed acetone extract contains potent biotherapeutic molecules having strong antioxidant, antibacterial, antifungal, antioxidant, anticancer and anti-inflammatory properties. Thus the *S.cumini* seed is a potent therapeutic moiety that can be further translated into a prototype for the development of chemotherapeutic drugs for ovarian cancer.

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References

1. Alghuthaymi M.A., Patil S., Rajkuberan C., Krishnan M., Krishnan U. and Abd-Elsalam K.A., *Polianthes tuberosa*-mediated silver nanoparticles from flower extract and assessment of their antibacterial and anticancer potential: an *in vitro* approach, *Plants*, **12(6)**, 1261 **(2023)**

2. Al-Nemari R., Al-Senaidy A., Semlali A., Ismael M., Badjah-Hadj-Ahmed A.Y. and Ben Bacha A., GC-MS profiling and assessment of antioxidant, antibacterial and anticancer properties of extracts of *Annona squamosa* L. leaves, *BMC Complementary Medicine and Therapies*, **20**, 1-14 **(2020)**

3. Al-Sarayreh Sajeda, Aljbour Salah H., Al-Harahsheh Adnan and Al-Hamaiedeh Husam, Enhancing Sodium Removal from Greywater using Modified Tripoli Adsorbents, *Res. J. Chem. Environ.*, **28(12)**, 1-7 **(2024)**

4. Azzaz N., Hamed S.E. and Mohamed A., Antimicrobial and anticancer activities of *Syzygium cumini* extracts, *Journal of Agricultural Chemistry and Biotechnology*, **13(3)**, 35-38 **(2022)**

5. Burki S., Burki Z.G., Jahan N., Muhammad S., Mohani N., Siddiqui F.A. and Owais F., GC-MS profiling, FTIR, metal analysis, antibacterial and anticancer potential of Monotheca buxifolia (Falc.) leaves, *Pakistan Journal of Pharmaceutical Sciences*, **32(5)**, 2405-2413 **(2019)**

6. Chandrasekaran R. et al, Assessment of anticancer properties of cumin seed (Cuminum cyminum) against bone cancer, *Frontiers in Oncology*, **13**, 1322875 **(2023)**

7. Chelghoum M., Guenane H., Tahri D., Laggoun I., Marfoua F.Z., Rahmani F.Z. and Yousfi M., Influence of altitude, precipitation and temperature factors on the phytoconstituents, antioxidant and α-amylase inhibitory activities of *Pistacia atlantica*, *Journal of Food Measurement and Characterization*, **15(5)**, 4411-4425 **(2021)**

8. Doonan F. and Cotter T.G., Morphological assessment of apoptosis, *Methods*, **44(3)**, 200-204 **(2008)**

9. Elnour A.A.M., Mirghani M.E.S., Musa K.H., Kabbashi N.A. and Alam M.Z., Challenges of extraction techniques of natural antioxidants and their potential application opportunities as anti-cancer agents, *Health Science Journal*, **12(5)**, 596 **(2018)**

10. Heinen A., Camara A.K., Aldakkak M., Rhodes S.S., Riess M.L. and Stowe D.F., Mitochondrial Ca2+-induced K+ influx increases respiration and enhances ROS production while maintaining membrane potential, *American Journal of Physiology-Cell Physiology*, **292(1)**, 148-156 **(2007)**

11. Karade P.G. and Jadhav N.R., *In vitro* studies of the anticancer action of *Tectaria cicutaria* in human cancer cell lines: G0/G1 p53-associated cell cycle arrest-Part I, *Journal of Traditional and Complementary Medicine*, **8(4)**, 459–64 **(2018)**

12. Kim J., Park E.Y., Kim O., Schilder J.M., Coffey D.M., Cho C.H. and Bast Jr. R.C., Cell origins of high-grade serous ovarian cancer, *Cancers*, **10(11)**, 433 **(2018)**

13. Kinghorn A.D., Pan L., Fletcher J.N. and Chai H., The relevance of higher plants in lead compound discovery programs, *Journal of Natural Products*, **74(6)**, 1539-1555 **(2011)**

14. Lanjewar A.M., Sharma D., Kosankar K.V. and Thombre K., Extraction and phytochemical screening of Syzygium cumini seeds in Vidarbha region of India, *World Journal of Pharmaceutical Research*, **7(5)**, 1782-91 **(2018)**

15. Matés J.M., Segura J.A., Alonso F.J. and Márquez J., Oxidative stress in apoptosis and cancer: an update, *Archives of Toxicology*, **86**, 1649-1665 **(2012)**

16. Meinhold-Heerlein I., Fotopoulou C., Harter P., Kurzeder C., Mustea A., Wimberger P. and Sehouli J., The new WHO classification of ovarian, fallopian tube and primary peritoneal cancer and its clinical implications, *Archives of Gynecology and Obstetrics*, **293**, 695-700 **(2016)**

17. Pereira J.M., Lopes-Rodrigues V., Xavier C.P., Lima M.J., Lima R.T., Ferreira I.C. and Vasconcelos M.H., An aqueous extract of *Tuberaria lignosa* inhibits cell growth, alters the cell cycle profile and induces apoptosis of NCI-H460 tumor cells, *Molecules*, **21(5)**, 595 **(2016)**

18. Prat J., FIGO Committee on Gynecologic Oncology., Staging classification for cancer of the ovary, fallopian tube and peritoneum, *International Journal of Gynecology & Obstetrics*, **124(1)**, 1-5 **(2014)**

19. Reid B.M., Permuth J.B. and Sellers T.A., Epidemiology of ovarian cancer: a review, *Cancer Biology & Medicine*, **14(1)**, 9 **(2017)**

20. Santucci C., Carioli G., Bertuccio P., Malvezzi M., Pastorino U., Boffetta P. and La Vecchia C., Progress in cancer mortality, incidence and survival: a global overview, *European Journal of Cancer Prevention*, **29(5)**, 367-381 **(2020)**

21. Singh D.B., Natural lead compounds and strategies for optimization, *Frontiers in Computational Chemistry*, **4**, 1-47 **(2018)**

22. Sophia A., Faiyazuddin M., Alam P., Hussain M.T. and Shakeel F., GC–MS characterization and evaluation of antimicrobial, anticancer and wound healing efficiency of combined ethanolic extract of *Tridax procumbens* and *Acalypha indica*, *Journal of Molecular Structure*, **1250**, 131678 **(2022)**

23. Sugiyama T. and Konishi I., Emerging drugs for ovarian cancer, *Expert Opinion on Emerging Drugs*, **13(3)**, 523-536 **(2008)**

24. Suroowan S. and Mahomoodally M.F., Tradition Meets Innovation: Herbal Medicine as a Sustainable Source of Anticancer Agents, In Urban Health Risk and Resilience in Asian Cities, Springer, Singapore, 367-387 **(2020)**

25. Vardhini S.P., Sivaraj C., Arumugam P., Ranjan H., Kumaran T. and Baskar M., Antioxidant, anticancer, antibacterial activities and GCMS analysis of aqueous extract of pulps of *Aegle marmelos* (L.) Correa, *The Journal of Phytopharmacology*, **7(1)**, 72-78 **(2018)**

26. Yadav S.S., Meshram G.A., Shinde D., Patil R.C., Manohar S.M. and Upadhye M.V., Antibacterial and anticancer activity of bioactive fraction of *Syzygium cumini* L. seeds, *HAYATI Journal of Biosciences*, **18(3)**, 118-122 **(2011)**

27. Zou Y., Kim D., Yagi M., Yamasaki Y., Kurita J., Iida T. and Oda T., Application of LDH-release assay to cellular-level evaluation of the toxic potential of harmful algal species, *Bioscience, Biotechnology and Biochemistry*, **77(2)**, 345-352 **(2013)**.

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