IN VITRO CYTOTOXICITY AND APOPTOSIS INDUCTION IN HUMAN OROPHARYNGEAL CARCINOMA KB CELLS BY PURIFIED ESTERASE ISOZYME EXTRACT OF URGINEA POLYPHYLLA HYACINTHACEAE.

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Abstract
Plant extracts which are natural products having potent anticancer activity is good for treating cancer. The use of plants for the treatment and prevention of cancer is giving much attention in the medical field. Biologically active compounds extracted from medicinal plants are currently being evaluated as source of promising anticancer agents. They are important for the discovery of new drugs for cancer treatment. The aim of the present study was to investigate the anticancer activity of Purified Esterase Isozyme isolated from Urginea species on KB carcinoma cell of human mouth (Oral cancer) We report the anticancer effects of Urginea Polyphylla, a species of Urginea collected from Castle rock Poona exhibited anticancer activity against cell line KB epidermal carcinoma (Oral cancer). This study confirms the potency of its cytotoxic activity on KB cancer cell lines by MTT ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The apoptosis-inducing activity by Annexin-V and Caspase 3 determination and its effect on mitochondrial membrane potential were measured by flow cytometry. Further DNA damage can be explained by TUNEL assay.

Key words: KB Cancer cell lines, Cytotoxicity, MTT, Annexin, Apoptosis, TUNEL, Caspase 3, flow cytometry

1. Introduction
Plant products are found to be effective and safe. Plant extracts which are natural products having potent anticancer activity is good for treating cancer. Today many plants with anti-cancer activity have been discovered. Since ancient times plants have been used for health benefits the traditional therapy involves the use of plants active principles which are beneficial to combat cancer. The search for plant drug sources started in 1950’s and they proved effective as anticancer agents. Cancer cells invade and destroy normal cells. This occurs due to imbalance in the body. The wild onion Urginea shares the properties of cultivated onion Allium cepa. Since Maharshi Athreya and Dhanavantri period Urginea has been used as an anticancer agent. Hence this plant has been selected for our studies. Natural products are safe, and some have the capability of reducing the mutagenicity in normal cells.

The genus Urginea is a medicinal plant that grows in India, Africa and Mediterranean regions of the world. Chemotherapeutants affect the hosts normal cells severely. Biologically active compounds are important for the discovery of new drugs for cancer treatment. The Bioactive molecules extracted from Urginea species i.e U. indica, U. polyphylla and U. wightii. Among three species U. indica has already proved to be anticancerous and it is an additional support to the efforts of National cancer institute to cure cancer [4]. The main problem in the cancer chemotherapy is the toxicity of the established drug. Medicines are available to various types of cancer, but no drug is found to be fully effective and safe. Plant products are found to be effective and safe. Scientists are investigating properties of medicinal plants in order to develop novel drugs against disease like cancer, from natural products. Medicinal herbs have profound
source and scope and have been used to find potential anticancer compounds in them [21]. Phytochemicals from medicinal plants can be used for Cancer treatment, may reduce adverse side effects, and help to treat cancer and shown promising anticancer efficiency against different cancers like human mouth epidermal carcinoma (KB cell line), murine leukemia (P388 cell line), human colorectal cancer (BE cell line) and prostate cancer in PC3 cell line [9]. The objective of this study was to examine the effect of Purified Esterase Isozyme isolated from Urginea polyphylla on KB carcinoma cell of human mouth (Oral cancer).

Esterases is a hydrolase enzyme. A wide range of different esterases exist that differ in their substrate specificity, their protein structure and biological function, it accelerates the hydrolysis and synthesis of Esters. They are widely distributed in plants, animals and microorganisms. Esterases are over expressed in cancerous cells and can have chiral specificity differing from that of the corresponding normal tissues. For this reason, Ester prodrugs could be promising approach in chemotherapy.

2. Materials and Method

2.1 Collection of Plants

The bulbs of Urginea polyphylla Hook. F. were collected from Poona and identified by the experts with the accession number 864, Latitude 18.728201, Longitude 73.540479 and Altitude (MSL)(m) 675 respectively. The enzyme purified and isolated from U. Polyphylla using the methods of ammonium sulphate salt precipitation, dialysis, ion exchange chromatography and gel filtration chromatography. Ion exchange chromatography fractions from Urginea polyphylla exhibited anticancer activity against cell line KB epidermal carcinoma (Oral cancer).

2.2 Cancer cell line

Human oropharyngeal carcinoma KB cells were incubated in Dulbecco’s modified Eagle medium with high glucose (DMEM-HG) supplemented with 10% Foetal Bovine Serum (FBS).

2.3 MTT/cytotoxicity assay against cancer cell lines

MTT/cytotoxicity assay against cancer cell lines Cytotoxic activity of plant extract was evaluated in 96-well flat-bottomed micro-titer plates by using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyltetrazolium bromide) assay [1]. For this purpose Dulbecco's Modified Eagle Medium with High Glucose (DMEM-HG) supplemented with 10% Foetal Bovine Serum (FBS), 1X Dulbecco’s Phosphate Buffered Saline (DPBS), 0.25% Trypsin-EDTA solution, MTT reagent, Dimethyl Sulfoxide (DMSO) from HiMedia, India were used and Cell culture treated T-25 flasks from Biolite, Thermo Fisher Scientific Inc., USA, 10mL serological pipettes and 96-well plates from Nunc, Thermo Fisher Scientific Inc., USA, 5mL, 2mL and 1.5mL tubes, Tarsons, India were used for the assay.

Cells cultured in T-25 flasks were trypsinized and aspirated into a 5mL centrifuge tube. Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted, using DMEM HG medium, such that 200μl of suspension contained approximately 10,000 cells. To each well of the 96 well microtitre plate, 200μl of the cell suspension was added and the plate was incubated at 37˚C and 5% CO2 atmosphere for 24 h. After 24 hours, the spent medium was aspirated. 200μl of different test concentrations of standard drug Cisplatin (5, 10, 20, 30 and 40 μg/mL from stock) and test drugs (0.05, 0.1, 0.2, 0.4 and 0.8 %v/v diluted from stock) were added to the respective wells. The plate was then incubated at 37˚C and 5% CO2 atmosphere for 24 h.

The plate was removed from the incubator and the drug containing media was aspirated. 200μl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/mL and the plate was incubated at 37˚C and 5% CO2 atmosphere for 3 h.

The culture medium was removed completely without disturbing the crystals formed. Then 100μl of solubilisation solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm and also at 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of test drug needed to inhibit cell growth by 50% (IC50) was generated from the dose-response curve for the cell line.

2.4 Annexin V Apoptosis Assay by Flow cytometry

The reagents used for Annexin V Apoptosis assay are BD Biosciences FITC Annexin V Apoptosis Detection Kit I - BD, Catalog no. 556547, FITC Annexin V (component no. 51-65874X), Propidium Iodide (PI)
(component no. 51-66211E) is a convenient, ready-to-use nucleic acid dye, 10X Annexin V Binding Buffer (component no. 51-66121E): 0.1 M Hepes/NaOH (pH7.4), 1.4 M NaCl, 25 mM CaCl2. For a 1X working solution, diluted 1 part of the 10X Annexin V Binding Buffer to 9 parts of distilled water. 5ml sterile centrifuge tubes - Tarsons, India. 1x phosphate-buffered saline (PBS) - HiMedia and Trypsin-EDTA solution - HiMedia were used [13].

Cultured cells in a 6-well plate at a density of 3 x 105 cells/2 ml and incubated in a CO2 incubator overnight at 37°C for 24 hours. Aspirated the spent medium and washed with 1ml 1X PBS. Treated the cells with required concentration of experimental test compounds and control in 2 ml of culture medium and incubate the cells for 24 hours. Left one of the wells as untreated to be used as negative control. Aspirated the medium and washed with 1ml 1X PBS. Treated the cells with required concentration of toxic compound in 2 ml of culture medium and incubated the cells for 12-15 hours. At the end of the treatment, removed the medium from all the wells into 5ml centrifuge tubes and wash with 500 µl PBS (Removed the PBS and added 200 µl of trypsin-EDTA solution and incubated at 37°C for 3-4 minutes. Pour the culture medium back into their respective wells and harvested the cells directly into the centrifuge tubes. Centrifuged the tubes for five minutes at 300 x g at 25°C. Carefully decanted the supernatant. Washed with PBS twice. Decanted the PBS completely. Resuspend cells in 1X Binding Buffer at a concentration of 1 x 106 cells/ml. Transferred 100 µl of the solution (1 x 105 cells) to a 5 ml culture tube. Added 5 µl of FITC Annexin V. Gently vortex the cells and incubated for 15 min at RT (25°C) in the dark. Added 5 µl of PI and 400 µl of 1X Binding Buffer to each tube and vortex gently. Analyse by flow cytometry immediately after addition of PI.

2.5 Flow cytometry and Caspase-3 expression
Cultured cells in a 6-well plate at a density of 3 x 10^5 cells/2 ml and incubate in a CO2 incubator overnight at 37°C for 24 hours [8]. Aspirated the spent medium and washed with 1ml 1X PBS. Treated the cells with required concentration of experimental test compounds and control in 2 ml of culture medium and incubated the cells for 24 hours. Left one of the wells as untreated to be used as negative control. At the end of the treatment, removed the medium from all the wells into 5ml centrifuge tubes and wash with 500 µl PBS. Removed the PBS and add 200 µl of trypsin-EDTA solution and incubated at 37°C for 3-4 minutes. Poured the culture medium back into their respective wells and harvested the cells directly into the centrifuge tubes. Centrifuged the tubes for five minutes at 300 x g at 25°C. Carefully decanted the supernatant. Washed with PBS twice. Decanted the PBS completely. Resuspend cells in 1X Binding Buffer at a concentration of 1 x 106 cells/ml. Transferred 100 µl of the solution (1 x 105 cells) to a 5 ml culture tube. Added 5 µl of FITC Annexin V. Gently vortex the cells and incubated for 15 min at RT (25°C) in the dark. Added 5 µl of PI and 400 µl of 1X Binding Buffer to each tube and vortex gently. Analyse by flow cytometry immediately after addition of PI.

2.6 Detection of DNA Damage by TUNEL assay
TUNEL Protocol:
Suspended the cells in 1% (w/v) paraformaldehyde in PBS (pH 7.4) at a concentration of 1-2 x 106 cells/ml. Placed the cell suspension on ice for 30-60 minutes. Centrifuged the cells for 5 min at 300 x g and discarded the supernatant [22].
Washed the cells in 5 ml of PBS, then pellet the cells by centrifugation. Repeat the wash and centrifugation. Resuspend the cell pellet in the residual PBS in the tube by gently vortexing the tube. Adjusted the cell concentration to 1-2 x 106 cells/ml in 70% (v/v) ice cold ethanol. Let cells stand for a minimum of 30 minutes on ice or in freezer. See note below. Stored cells in 70% (v/v) ethanol at -20°C until use. Cells can be stored at -20°C several days before use.

Staining Protocol
The following protocol describes the method for measuring apoptosis in the positive and negative control cells that are provided in the APO-DIRECT™ Kit. First Resuspend the positive and negative control cells by swirling the vials. Removed 1 ml aliquots of the control cell suspensions (approximately 1 x 106 cells/ml) and placed in 12 x 75 mm centrifuge tubes. Centrifuge the control cell suspensions for 5 min at 300 x g and
removed the 70% (v/v) ethanol by aspiration, with proper care to not disturb the cell pellet. Resuspend each tube of control cells with 1.0 ml of Wash Buffer for each tube. Centrifuged as before and removed the supernatant. Repeated the Wash Buffer treatment. Resuspend each tube of the control cell pellets in 50 µl of the DNA Labeling Solution (prepared as described below). Incubated the cells in the DNA Labeling Solution for 60 min at 37°C. The reaction can also be carried out at RT overnight for the control cells. For test samples, the 60 min incubation time at 37°C may need to be adjusted to longer periods of time. At the end of the incubation time, add 1.0 ml of Rinse Buffer to each tube and centrifuge each tube at 300 x g for 5 min. Remove the supernatant. Repeated the cell rinsing with 1.0 ml of the Rinse Buffer, centrifuge, and remove the supernatant. Resuspend the cell pellet in 0.5 ml of the PI/RNase Staining Buffer. Incubated the cells in the dark for 30 min at RT. Analyzed the cells in PI/RNase solution by flow cytometry. Analyzed the cells within 3 hours of staining. Cells may begin to deteriorate if left overnight before analysis.

**3. Result and Discussion**

Cancer kills several million people all around the world. Chemotherapeutic agents used in cancer treatment is toxic and their usage is banned. American cancer society detected cancer patients and recorded 2 to 31 % annual deaths. Thus, cancer is a major issue in developed and in undeveloped countries.

Mutations in DNA leads to cancer. Factors that cause cancer are smoking, diet, chemicals, radiations and pollutants in food, water and air. The main disadvantages of synthetic drugs are side effects. Cancer being associated with high rate of mortalities, the plant drugs without side effects would be a great relief to the sufferer of cancer.

The search for new anti- cancer drug is one of the most prominent research areas of natural products, the cytotoxicity activity was evaluated on human oropharyngeal carcinoma KB cells. Cytotoxic effect of esterase an isozyme isolated and purified from *Urginea polyphylla* on KB carcinoma cells were determined using MTT assay and also Annexin V, apoptosis assay by flow cytometry, Caspase-3 expression by flow cytometry and detection of DNA damage by tunnel assay and. the results obtained are as follows.

The extract from *Urginea polyphylla* (Esterase) exhibited promising activity against the KB carcinoma cell line with survival of less than standard at the concentration of 50µg/ml. The esterase showed potent cytotoxic activity with mean IC50 value of 0.136 %v/v in crude extract and 0.261%v/v in purified extract respectively (Table-1). Both crude and purified compounds possessed high activity against KB cell line. Hence the extract purified esterase from *Urginea polyphylla* appears to be the most potent and highly selective against carcinoma.

Ic50 values are the concentration of extracts required to inhibit 50% of cell growth and proliferation, respectively. Ic50 value of standard cisplatin used is 6.218 µg/mL.

IC50 value determines the level of potency of a compound in inhibiting cell growth, the lower value of the ic50 indicate higher potential of a compound inhibiting cell growth. The composition of the extract differs depending on the solvent used. The extract isolated and purified from *Urginea polyphylla* reduced the variability of KB carcinoma cells.

**Table-1: IC50 value of Standard and Urginea samples for KB cell line for 24-hour treatment**

<table>
<thead>
<tr>
<th>Sample name</th>
<th>KB cell line IC50 24hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin (Standard)</td>
<td>6.218 µg/mL</td>
</tr>
<tr>
<td>Crude 1 (<em>Urginea indica</em>)</td>
<td>1.129 %v/v</td>
</tr>
<tr>
<td>Crude 2 (<em>Urginea wightii</em>)</td>
<td>NA - No significant toxicity</td>
</tr>
<tr>
<td>Crude 3 (<em>Urginea polyphylla</em>)</td>
<td>0.136 %v/v</td>
</tr>
<tr>
<td>Purified 1 (<em>Urginea indica</em>)</td>
<td>NA- Repeated contamination in sample</td>
</tr>
<tr>
<td>Purified 2 (<em>Urginea wightii</em>)</td>
<td>NA- Repeated contamination in sample</td>
</tr>
<tr>
<td>Purified 3 (<em>Urginea polyphylla</em>)</td>
<td>0.261 %v/v</td>
</tr>
</tbody>
</table>

Three species of *Urginea; Urginea indica* (Sample 1), *Urginea wightii* (Sample 2) and *Urginea polyphylla* (sample 3) crude and purified fractions were taken for MTT assay analysis in which we found out *Urginea polyphylla* purified fraction showed potential toxicity whereas *Urginea indica* and *Urginea wightii* purified fraction did not show significant toxicity. Graphical representation of *Urginea polyphylla* crude and purified sample and standard Cisplatin verses KB cell lines showing cytotoxicity are depicted below (Graph 1-3).
Graph-1: Graphical representation of KB cell line VS Cisplatin

Graph 1: Graphical representation of KB cell line VS Cisplatin

\[ y = -25.09 \ln(x) + 95.851 \]
\[ R^2 = 0.7073 \]

Graph-2: Graphical representation of KB cell line VS crude sample *Urginea polyphylla*

Graph 2: Graphical representation of KB cell line VS crude sample *Urginea polyphylla*

\[ y = -15.69 \ln(x) + 18.682 \]
\[ R^2 = 0.5993 \]
Graph-3: Graphical representation of KB cell line VS purified *Urginea polyphylla*

**KB Cell line Vs. Purified sample *Urginea Polyphylla***

\[
y = -37.86\ln(x) - 0.8835 \\
R^2 = 0.9478
\]

Apoptosis is a programmed cell death that removes unwanted or dead cells. Characteristics of apoptotic cells include condensation of the cytoplasm and nucleus. Aggregation of chromatin and formulation of membrane bound vesicles known as apoptotic bodies. Necrosis is a pathological activity represented by swelling of the cells that is accompanied by chromatin condensation. Necrotic cells show cellular structure and nuclear lysis along with subsequent inflammation. Signs of cell death were observed 24 hours after treatment with *Urginea polyphylla* (Esterase). Morphological changes include reduction in the size of the cells and the cells gradually become flat and shrunken with the appearance of small vesicle bodies (apoptotic bodies). Later after 72-hour incubation the sign of apoptotic activity was observed.

Apoptotic activity is usually associated with DNA cleavage. Annexin v apoptotic assay reveals that counting the percentage of cells in untreated sample live cells 88% and early apoptotic is 9.98% and late apoptotic is 1.88%. The same sample treated with Cisplastin standard live cells 83.4%, early apoptotic cells 13.9 % and late apoptotic cells 2.52% with geometric mean fluorescence 7398 While in the sample treated with purified esterase live cells 81.3%, early apoptotic 16.3 % and late apoptotic 2.14% with geometric mean fluorescence 7010, indicating that purified sample is showing more early apoptotic activity than the standard. In late apoptotic there could be both apoptotic and necrosis cells. (Table-2). The schematic representation showing the early and late apoptotic cells of *Urginea polyphylla*, standard drug Cisplastin and Untreated were shown in Fig-1-2.
Table-2: Annexin V Apoptosis assay showing percentage of apoptotic cells by flow cytometry

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample Name</th>
<th>% of Cells</th>
<th>Geometric mean fluorescence intensity (MFI) of FITC Annexin V (FL1-A parameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Live</td>
<td>Early Apoptotic</td>
</tr>
<tr>
<td>1</td>
<td>Untreated</td>
<td>88.0</td>
<td>9.98</td>
</tr>
<tr>
<td>2</td>
<td>Standard drug ‘Cisplatin’ - 6 μg/mL</td>
<td>83.4</td>
<td>13.9</td>
</tr>
<tr>
<td>3</td>
<td>Test Sample ‘Purified 3’ - 0.261 %v/v</td>
<td>81.3</td>
<td>16.3</td>
</tr>
</tbody>
</table>

Fig-1: Annexin V showing early and late apoptotic cells in *Urginea polyphylla* purified sample (A) and Standard cisplastin (B)

Fig-2: Annexin V showing early and late apoptotic cells in untreated
The multi parametric apoptosis assay is designed to differentiate apoptosis cells from living cells and necrotic cells in a single population. A distinctive feature of the early stages of apoptosis is the activation of caspase enzyme which participated in the cleavage of protein substrate and in the subsequent disassembly of the cells. A series of caspase assays are available that allow to detect active caspases in living cells by flow cytometry.

The cell event Caspase 3 flow cytometric detection of activated caspase 3 in apoptotic cells using novel fluorogenic caspase-3 detection reagents. During apoptosis caspase3 proteins are activated and able to cleave caspase 3 recognition sequence encoded in the DEVD peptide. Cleavage of the DNA by the reagent labels the apoptotic cells with a bright fluorescence signals that has absorption for the green detection reagent. We can distinguish apoptotic cells from live and necrotic cells. After flow cytometry analysis annexin V, positive and negative apoptotic populations were sorted and measured. Caspase family of Cystine proteases plays a key role in apoptosis and inflammation. Caspase-3 is a key protease that is processed in cells undergoing apoptosis by self-proteolysis. The processed form of caspases consists of large (17-22kDa) and small (10-12 kDa) subunits which associate to form an active enzyme. Active Caspase -3 is marker for cells undergoing apoptosis and consists of hetero dimer of 17 and 12kDa subunits derived from 32 kda pro enzyme. Active caspase 3 prototypically cleaves and activates other caspase as well as relevant target in the cytoplasm. The percentage of cells showing caspase 3 positive activity in standard is 19.5 % and in purified esterase sample 15.5 % with geometric mean fluorescence 2361 and 2220 respectively and percentage of negative caspase 3 in cisplatin is 80.5 % and purified Esterase sample 84.5%. (Table-3).

Table-3: Caspase 3 expression by flow cytometry

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Sample Name</th>
<th>Geometric fluorescence intensity (MFI) of FITC (Caspase-3 parameter)</th>
<th>% of Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Untreated</td>
<td>1312</td>
<td>95.5</td>
</tr>
<tr>
<td>2</td>
<td>Standard drug ‘Cisplatin’ - 6 µg/mL</td>
<td>2361</td>
<td>80.5</td>
</tr>
<tr>
<td>3</td>
<td>Test Sample ‘Purified 3’ - 0.261 %v/v</td>
<td>2220</td>
<td>84.5</td>
</tr>
</tbody>
</table>
DNA damage was detected by TUNEL assay which took 24 hours. In untreated the healthy cells counted was 97% and the DNA damaged is 3.05% with geometric mean fluorescence 1140. While in cisplatin the standard drug activity on KB cell line 53.9% healthy cells and 46.1% damaged DNA with a geometric mean fluorescence of 1889. In purified esterase treatment to KB cells healthy cell noticed was 52.1% and damaged DNA cells 47.9% with geometric mean fluorescence 1914. (Table-4) The purified sample is equally working like the standard drug and damaging DNA of cancerous cells. Thus, there is an increase in the DNA fragmentation and increase in overall mean fluorescence intensity was observed in our purified esterase obtained from *U. polyphylla* as compared to the control sample. (Fig:5).
Table-4: Detection of DNA damage by TUNEL assay

<table>
<thead>
<tr>
<th>Sample</th>
<th>% of Cells</th>
<th>Geometric Mean fluorescence intensity (MFI) of FITC-dUTP (FL1-A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy</td>
<td>Damaged DNA</td>
</tr>
<tr>
<td>1 Untreated</td>
<td>97.0</td>
<td>3.05</td>
</tr>
<tr>
<td>2 Standard drug ‘Cisplatin’ - 6 µg/mL</td>
<td>53.9</td>
<td>46.1</td>
</tr>
<tr>
<td>3 Test Sample ‘Purified 3’ - 0.261 %v/v</td>
<td>52.1</td>
<td>47.9</td>
</tr>
</tbody>
</table>

Fig-5: TUNEL assay showing healthy and damaged DNA in purified Urginea polyphylla sample(A), Standard cisplastin (B) and Untreated (C)

The bioactive compounds isolated and characterized from plants are now being used as therapeutic agents. A number of such molecules is under clinical studies. A synthetic flavone, favopiridol plant alkaloid isolated from Dysoxylum bineetariferum is showing its activity against tumors, leukemia and solid tumors [7]. Olomucine is a natural product isolated from Raphanus sativus has converted into a synthetic agent
roscovitine and it is in clinical trials in Europe [15]. Betulinic acid isolated from Zizyphus species; Z. mauritiana and Z. rugosa exhibited toxicity against human melanoma cell lines [5,18]. Silvestrool was isolated from the fruits of Meliaceae family member Aglaila sylvestre showed cytotoxicity against breast and lung cancer cells [16].

Epothilones are bacteria macrolides, with potent antiproliferative activity which has lead structures for the discovery of several clinical candidate for cancer treatment. Several Epothilones have been advanced to clinical evaluation in humans. One has been approved by FDA in 2007 for clinical use. In breast cancer [2]. Studies also investigated on anticancer potential, phytochemical, anti-microbial and antioxidant study of Allium wallichii [11].

The esterase isolated and purified U. polyphylla has every potential to decrease growth of KB carcinoma an oral pharyngeal cancer. It has been reported that medicinal plants have rich anticancer potential and plays a significant role in curbing cancer and selected plants have been explored for biological activity and anticancer activity and the bioactive molecules isolated can be used to reduce the side effects associated with cancer. Patient suffering from cancer. Review on anticancer potential of plants and natural products explained how effective and safe natural products in the treatment and management of cancer [19]. There are guidelines for the selection of functional assays to evaluate the source of cancer also provided a link between available techniques and recognized hallmarks of cancer in their review have provided a link between available techniques and recognized hallmarks of cancer in their review [20]. Studies also have reported Markers for terminally differentiated chondrocytes, such as alkaline phosphatase, annexin II, annexin V and type X collagen and were analyzed the Immunohistochemical assay of human normal and osteoarthritic knee cartilage from medial and lateral femoral condyles [12]. Tunnel labeling used in these specimens to detect apoptosis. Susana et al., 2007 detected ALP isoenzymes expression by RT-PCR and Western blotting for Ki67 and TUNEL, assay was used for determining Cell proliferation and apoptosis. Specific isozyme of 2,5'-oligoadenylate synthetase, has an additional activity which promotes apoptosis in mammalian cells [3]. They have identified an interferon induced dual function protein of the Bcl2 family that can synthesize 2',5'-oligoadenylate synthetase and promote cellular apoptosis independently. Some researchers have studied Ribonucleotide Reductase as a Mechanism of resistance in a human oropharyngeal cancer KB cell line and the expressions of Ribonucleotide Reductase (RR) and deoxycytidine kinase (DCK) [25]. Curcumin has enormous potential in the prevention of cancer in both preclinical and clinical level. The stem part having potential to suppress proliferation of a wide variety of tumor cells [6]. Glutathione S-transferase (GST) activity and isozyme composition in nine human cell lines in which in majority of cases overall GST activity was higher in the tumors than in the cell lines [4]. The ethyl acetate (EtOAc) fungal culture extract isolated from Datura metel L showed cytotoxic activity against all the tested human cancer cell lines, against cervical cancer cells HeLa. [10]. Multidimensional approach to analyzing apoptosis gives more information than single-parameter assays. Telford et al. reported simultaneous analysis of caspase activation, annexin V binding to “flipped” phosphatidylserine residues and membrane permeability to DNA binding dyes [26]. Three medicinal plant Brucea javanica, Azadirachta indica, and Typhonium flagelliforme which are commonly used to treat conditions associated with tumor formation studied to determine Apoptosis-Inducing Effect on KB and ORL-48 oral cancer cell line in which Brucea sp. Extract showed more cytotoxicity activity [17]. Aqueous extracts prepared from six South African medicinal plants and studied for their cytotoxicity against three human cancer cell lines: DU-145 prostate cancer cells, MDA-MB-231 and MCF-7 breast cancer cells and a nonmalignant breast cell line, MCF-12A. α-Santalol derivatives were isolated from Santalum album heartwood [23,27]. Fourteen compounds were evaluated for their cytotoxicity against HL-60 and TIG-3 cells and (9S,10E)-9-hydroxy-α-santalal showed tumor selective cytotoxicity. It also induced caspase-dependent apoptotic cell death in HL-60 cell. Numerous factors are causing this multistep process, so it is essential to know the hallmark for cancer causing malignant transformation and progression. Studies have been reported most frequently used in vitro assays for analyzing each hallmark in cell culture. Also, hallmarks involving the immune system because of the difficulty in assessing them in cell culture [20].
4. Conclusion

In our studies we have used four assays to determine the activity of Esterase against KB carcinoma cells such as cytotoxicity by MTT assay, Annexin V apoptosis assay by flow cytometry, detection of DNA damage by TUNEL assay, Caspase-3 expression by flow cytometry. In this study we have compared the apoptosis inducing capacity of the sample with the standard drug cisplatin and the test samples have shown good activity in comparison to cisplatin so this can be studied further for their therapeutic potential in treatment of cancer. but it’s better to evaluate the cytotoxic effects of natural product for this we needed to carry out invivo and invitro tests and whether the bioactive compounds are safe to use and at which concentration is very much necessary. So, it is documented that Urginea, medicinal herbs have richest source of anticancer potential can play significant role in clinical application. Cancer being associated with high mortality rates if herbs can be used even in the palliative care or to reduce the side effects associated with cancer would be of great relief for the sufferer. Urginea polyphylla showed the most pronounced cytotoxic activity, will be evaluated further for the possible isolation of active compounds.

References


