

Original Research Article

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## Effect of *Sida cordata* (Burm.F.) Borss Extracts on Oral Cancer: An *invitro* Study

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### ABSTRACT

#### Keywords

Antiproliferative, Apoptosis, Human tongue squamous cell carcinoma (CAL-27)

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Natural components of the plants, animals and mineral sources are used as medicines since ancient time as they have fewer or no side effects, plant products have been widely used in controlling cancer as complementary to synthetic medicine is gaining increased popularity. Present study was carried out with different extract of leaf, stem, root and the whole plant of *Sida cordata* (burm.f.) Borss (SC) to determine their probable anticancer/ antiproliferative effect on oral cancer using Human tongue squamous cell carcinoma (CAL-27) cell lines and normal human gingival fibroblast (HGF) cell lines in comparison with commonly used standard drug Cisplatin. Among the various extracts used in the study, leaf extracts significantly reduced the cell viability, Highest inhibition was recorded in the ethanol extract (87.5%) followed by water (85%) at 200µg/min the MTT assay carried out. Further TUNEL assays revealed that SC extracts induces the apoptosis.

### Introduction

Cancer is one of the major causes of mortality as per the reports available globally. The numbers of cancer cases are increasing gradually. Several medicines are available commercially for the treatment of various types of cancers but no drug is found to be completely effective and safe. The major problem in the cancer treatment is the side

effects of chemotherapy, however plants and plant derived products have proved effective and safe in the treatment and management of cancer.

Efforts are going on to find out natural agents for cancer treatment which may minimize many side effects of radiotherapy and chemotherapy treatments. Oral cancer is the sixth most common cancer affecting mankind

as per the WHO reports, among all the malignancies oral cancer has the highest mortality rate (Stewart, and Wild, 2014). Present oral cancer treatment is based on the classical methods such as surgery, radiation, chemotherapy or a combination of these methods (Saman, 2012). The synthetic drugs used in chemotherapy not only destroys cancer cells, stop their proliferation, spreading, shrinkage of tumor or relieving the cancer symptoms, but they do also destroy or slow down the growth of normal cells, mainly the cells of the hair, mouth, digestive system, as well as blood cells (Hsu S, *et al.*, 2004). Commonly used chemotherapy drug is Cisplatin combined with other cancer drugs. It is found effective in patient with advanced squamous cell oral cancer (Lajimi AA, *et al.*, 2010), response and its various side effects varies from persons to persons therefore oncologists are still searching for new anticancer drugs with more potent inhibitory and less side effects (Andreadis *et al.*, 2003). It is conceivable that effective plant-derived chemoprevention agents might target molecules that regulate the cell cycle, cellular senescence, and apoptosis. In the present study an attempt is being made to identify the anti-cancer activity in the plant *Sida cordata* (burm.f.) Borss.

*Sida cordata* (burm.f.) Borss. The member of Malvaceae family, is a small weed found throughout India, usually on the road sides and other waste places. A procumbent, diffuse, much branched hairy herb with a very short main stem and long slender trailing branches that occasionally root at places of contact with the soil. Leaves are long-petioled, cordate to roundish with stellate hairs, flowers are yellow, solitary or in pairs in the axils, fruits is schizocarp located within the persistent calyx, seeds brownish, glabrous (Paul and Nayar, 1988). It is used as a medicinal source in the codified Indian systems of medicine like Ayurveda and

Siddha. In ayurvedic system of medicine it is widely used as antibacterial, antitumor, antifungal, antiulcer, antitussive, anti-inflammatory, anti-malarial, antioxidant, analgesic, anti-depressant, anti-hyperglycemic, hepato protective agent (Bhava Mishra *et al.*, 2009; Panthi *et al.*, 2009). However, the anti-cancerous properties of SC are hitherto unknown. Therefore present study was planned to find out its anti-cancerous activity. Our earlier studies on antimicrobial, anthelmintic activity and phytochemical analysis of different extracts of *Sida cordata* (Burm.f.) Borssum leaf, stem and root and the whole plant (Gulnaz and Savitha, 2013; Gulnaz and Savitha, 2015; Gulnaz *et al.*, 2018) had proved its efficacy which validates it as a traditional medicine to cure various diseases, hence present study was extended to give a scientific validation to traditional use as a source of medicine to cure oral cancer.

## **Materials and Methods**

### **Plant collection**

The Fresh plant *Sida cordata* (Burm.f.) Borssum was collected from its natural habitat from the forest region of Somawarpet in Madikeri, Kodagu district Karnataka. The plant was identified and authenticated at National Ayurveda Dietetics Research Institute Bangalore, (voucher no: RRCBI-11748). The plants were washed thoroughly under tap water, shade dried at room temperature and then homogenized to fine powder of 40mm mesh sizes and stored in airtight bottles at 4°C (Fig. 1).

### **Extraction of plant material**

The dried and powdered plant material were subjected to Soxhlet extraction by a hot percolation method (40-60°C) with different solvents (500ml each) in their increasing

order of polarity, the solvents used were petroleum ether (A), chloroform (B), ethyl acetate (C), 95% ethanol (D) and water (E). Each solvent extraction step was carried out until the extractive becomes colorless. The solvent was completely removed in each case before the next extraction. After extraction the extracts were concentrated by evaporation using flash evaporator and the dried extracts were weighed in air tight bottles and refrigerated until use.

### **Cytotoxicity assay**

Human tongue squamous cell carcinoma (CAL-27) cell lines and normal human gingival fibroblast (HGF) cells as a control to represent normal oral mucosa cells for comparative purposes was used. The cells were grown in DMEM supplemented with 10% FBS, 50 U/ml penicillin G, and 50 mg/ml streptomycin sulphate. The cultures were maintained at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Exponentially growing cells were used for all the experiments.

Cytotoxicity activity was evaluated by MTT assay which is based on the reduction of MTT by mitochondrial dehydrogenases of viable cells to a purple formazon product. Briefly CAL-27 cells were divided into 3 groups: untreated negative control, cisplatin treated as positive control, and the test group treated with different doses of SC extracts, cells are diluted in growth medium and seeded in 24-well plates. After overnight growth, the growth medium is replaced with exposure medium (DMEM without FBS) containing one ml of the different extract / standard solution in various concentrations (50-200 µg/ml) was added. Water is used in place of plant extract for negative control. After 24hrs the cells in each well was washed with 200 ml of PBS, and incubated with 100 ml of 500 µg /ml MTT in PBS at 37°C for 3

hrs. Same treatment was given to normal human gingival fibroblast (HGF) cells also. The MTT-formazon product formed is dissolved in 200 ml of DMSO and is estimated by measuring the absorbance at 570 nm in an ELISA plate reader. All the testes were carried out in triplicate Cell survival is expressed as percentage of viable cells of treated samples to control samples.

$\% \text{ Protection} = 100 - [(\text{Optical density of Test sample} / \text{Optical density of Control}) \times 100]$

### **Assessment of cell morphology in response to the extracts**

Morphological changes of the cells in response to different extracts was observed periodically and images were captured under an inverted microscope.

### **Immuno histochemical (IHC) staining**

#### **TUNEL assay**

The terminal deoxy nucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) technique was used to find out the presence of apoptotic cells. An in situ apoptosis detection kit was used in accordance with the manufacturer's procedure. CAL-27 and HGF cell lines were grown on chamber slides, treated with various SC extracts for 48 hrs. The cells were then fixed with 4 % paraformaldehyde for 10 min, and then incubated for 60 min with a reaction mixture containing fluorescein conjugated-dUTP and terminal deoxynucleotidyl transferase.

#### **DNA fragmentation analysis**

Gel electrophoresis was carried out to determine the band pattern of DNA fragments from extract treated CAL-27 cells lines. The Suicide-Track DNA Ladder Isolation kit was used.

## Results and Discussion

Herbal medicines prepared from medicinal plants have great importance in primary health care as they are free from side effects, and they are low cost medicines. Adverse effects of presently available synthetic drugs and effectiveness of newly developed plant medicines has led to resurgence of plant based remedies.

The percentage of yields (w/w) of various extracts is shown in Figure 2. The highest yield was recorded in aqueous medium and the order of yield was found to be the root  $\geq$  leaf  $\geq$  whole plant  $\geq$  stem (8.5%, 7.5%, 6.8% and 1.8% respectively) while with ethanol it was 5.8% for root, followed by whole plant, leaf and the stem. The result indicates water and ethanol serves as a good solvent for the extraction of bioactive compounds from the plant *Sida cordata* (burm.f.) Borss.

### Cytotoxicity / antiproliferative activity

Antiproliferative activity of various SC extracts and the standard Cisplatin is given in Figures 3.1-3.4. Antiproliferative activity was found to be directly proportional to the extract's concentration. In the present study all the leaf extracts showed antiproliferative activity in the range of 7.5 to 87.5% at the concentration of 50-200 $\mu$ g/ml (Fig. 3.1). Highest inhibition was recorded in the ethanol extract of leaf (87.5%) followed by water (85%) and 50% inhibition was seen at 5 and 10  $\mu$ g/ml respectively. Petroleum ether extracts exhibited lowest activity (32.5%) at 200 $\mu$ g/ml and 50% inhibition was seen at concentration of 100 $\mu$ g/ml in chloroform and ethyl acetate extracts. No significant antiproliferative activity was seen in all the stem extracts it was 0 to 13% at concentration of 5-200 $\mu$ g/ml (Fig. 3.2). In root extracts antiproliferative activity was in the range of 8 to 81% (Fig. 3.3).

Highest activity was recorded in Ethanol extract (81%) followed by water extract (78%) with 50% inhibition at 10 $\mu$ g/ml. Similar pattern was observed in whole plant also. Antiproliferative activity of the whole plant extracts ranged between 7.5 to 80% at the concentration of 50- 200 $\mu$ g/ml (Fig. 3.4). 50% of inhibition was noticed at 10 $\mu$ g/ml concentration for both ethanol and water extracts. For the standard drug Cisplatin antiproliferative activity was in the range of 49 to 83% at the concentration of 50-200 $\mu$ g/ml and 50% of inhibition was noticed at 8 $\mu$ g/ml

### Assessment of cell morphology in response to the extracts

Significant changes were observed in CAL-27 cells at different incubation period (24 hrs. -72 hrs.) after treating with 250 $\mu$ g/ml of different extracts of leaf, root and the whole plant of SC. Morphological changes observed were reduction in the size of the cells. Gradually cell flattening and shrinkage with the appearance of small vesicle bodies (apoptotic bodies) as shown in Figures 4 and 5, this indicates that death by apoptosis however there were no morphological changes observed in normal oral mucosa cells which strongly supports the extracts in the study under taken are safe and nontoxic to the normal cells.

### Immunohistochemically (IHC) staining

#### TUNEL assay

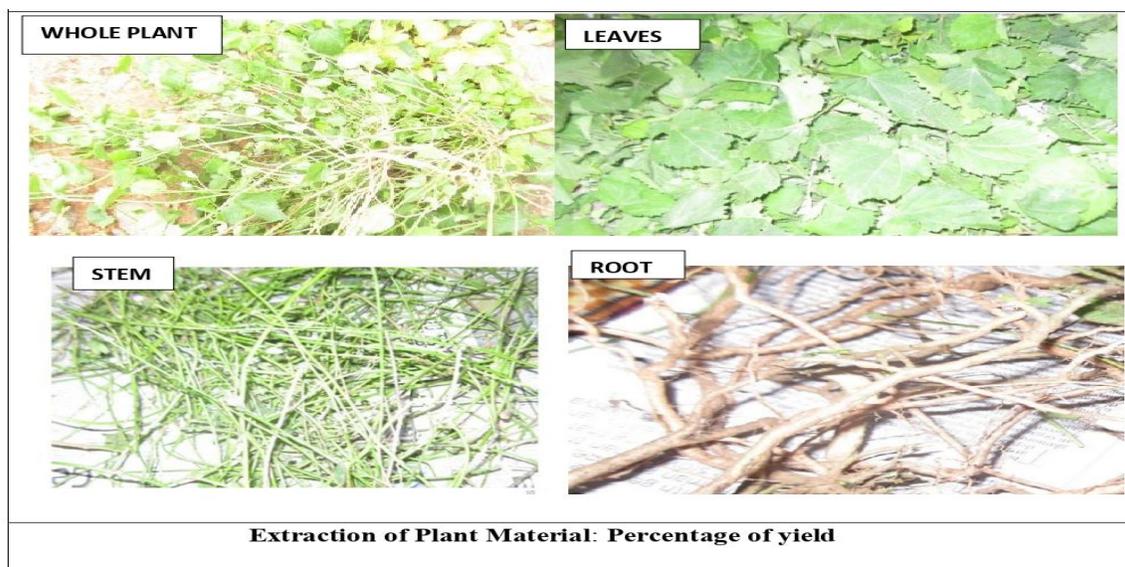
SC extracts induce the apoptosis of CAL-27 cell lines, to further confirm whether SC extracts induces apoptosis of CAL-27 cell lines, IHC was done. In which positively stained fluorescein-labeled cells were visualized and photographed using a fluorescence microscope. DAPI was used to visualize the nuclei the apoptotic cells were marked with higher fluorescein isothiocyanate

fluorescent intensity with green color. As shown in Fig. 6, SC extracts treatment significantly induces the apoptosis of CAL-27 cell lines,

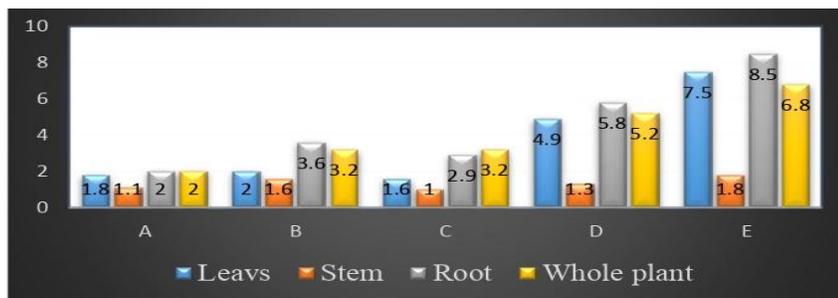
### DNA fragmentation analysis

Induction of apoptosis on CAL-27 cell lines by SC extracts was validated by DNA fragmentation analysis using gel electrophoresis technique. In CAL-27 cell lines treated with various SC extracts (0-200µg/ml concentration) ladder DNA band pattern was observed, lane (2 to 7) which indicates DNA has undergone fragmentation, Lane 1: negative control and each fragment corresponded to a band in the ladder. In this

study attempts were made to examine whether SC extracts have anti-cancerous properties on Human tongue squamous cell carcinoma CAL-27 cell lines. In MTT cell viability assays SC leaf extracts exhibited significant anti-proliferative effects on CAL-27 lines. Further TUENL assays supplemented that SC extracts induced the apoptosis in CAL-27 cell lines. These results support the notion that SC leaf extracts reduce the cell viability, and concomitantly activate the apoptosis. In normal oral mucosa cells no morphological changes were observed which strongly supports the extracts under in used in the study are safe and nontoxic to the normal cells (Fig. 7).

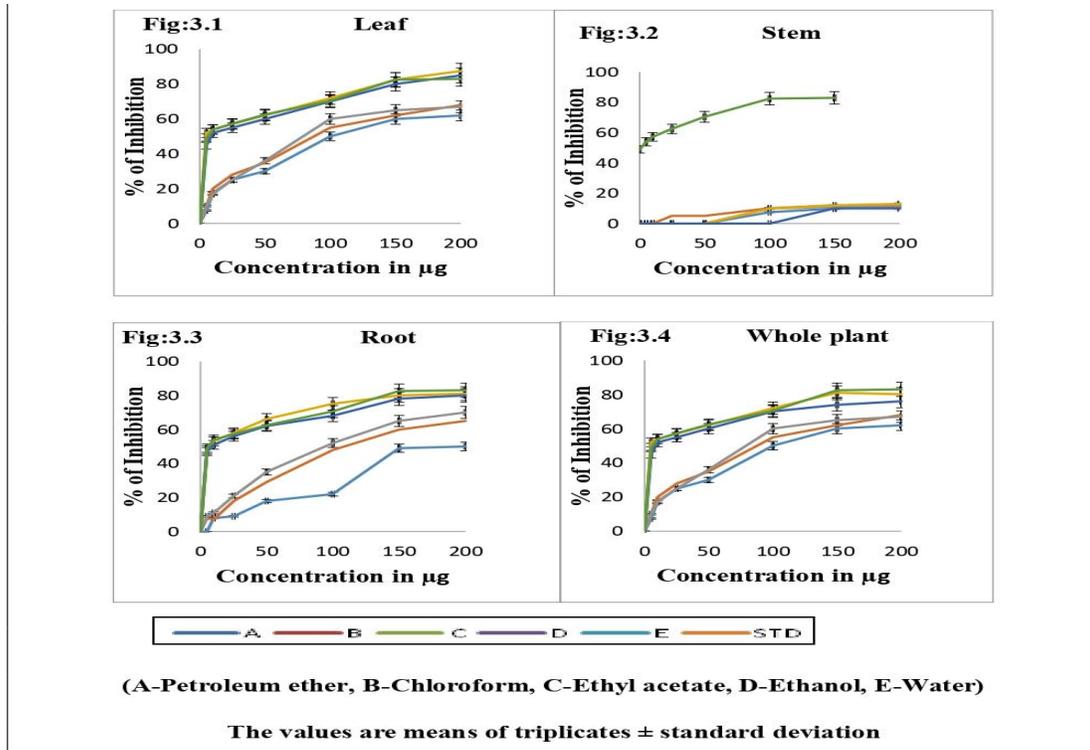


**Fig.1** Plant collection

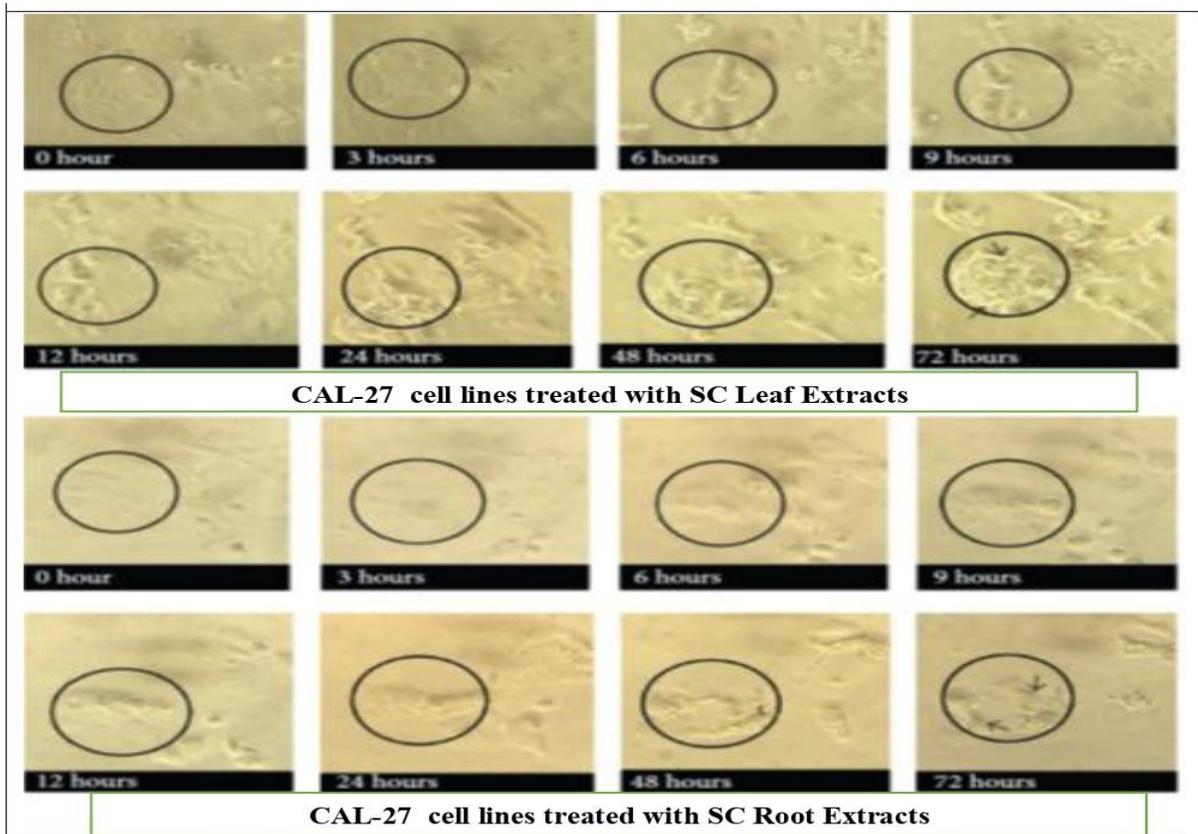


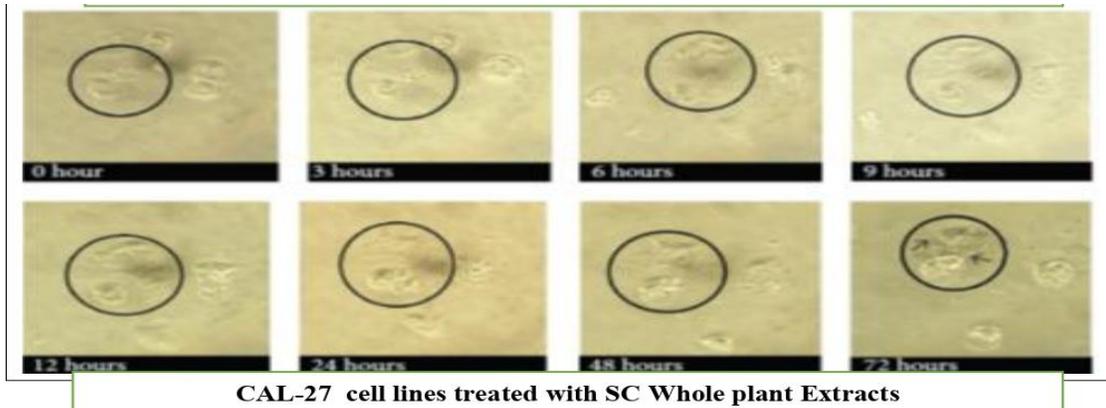
**(A-Petroleum ether, B-Chloroform, C-Ethyl acetate, D-Ethanol, E-Water)**

**Fig.2** Percentage of yields (w/w)

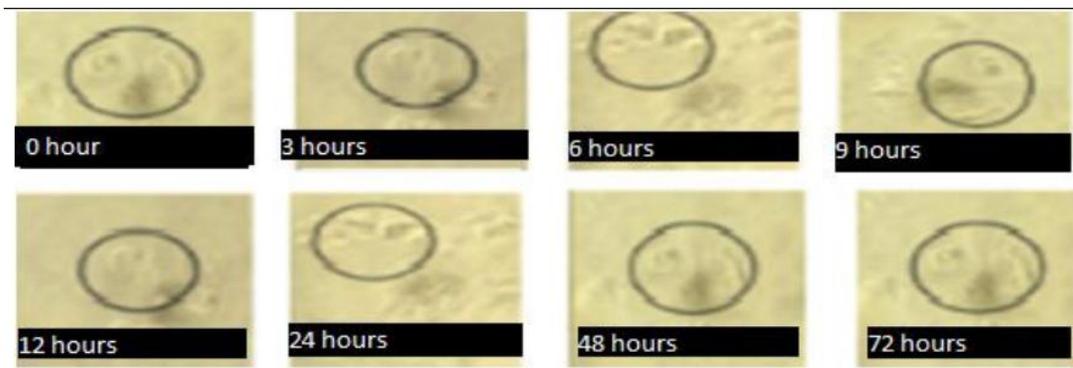


**Fig.3** Cytotoxicity/Antiproliferative activity by different extracts

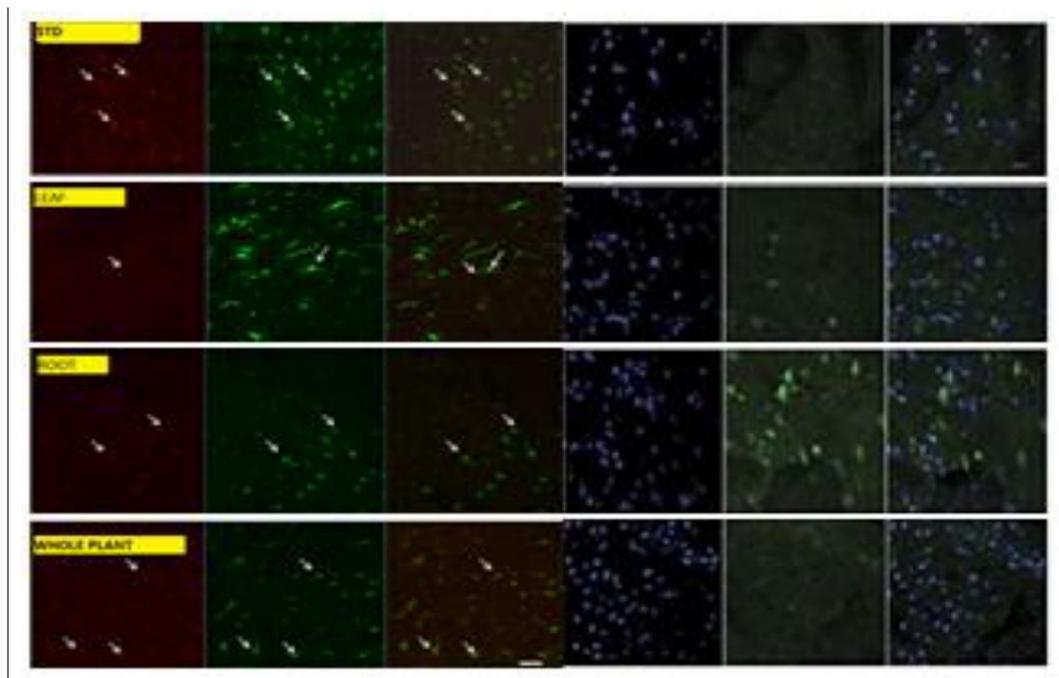




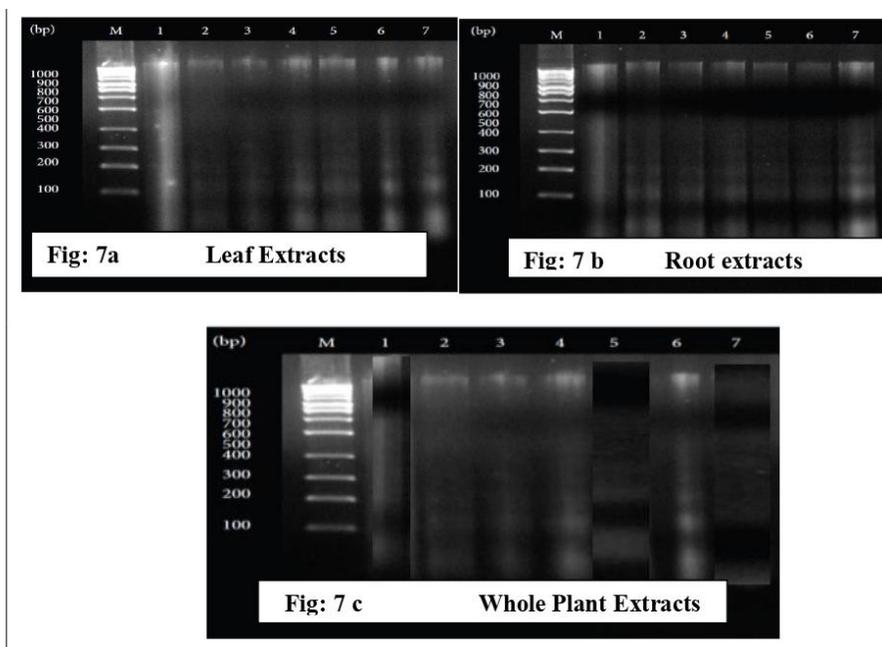
**Fig.4** Morphological Changes of CAL-27 cell lines in Response to extracts



**Fig.5** Morphological Changes HGF cell lines response to leaf extracts



**Fig.6** Immunohistochemical (IHC) staining



**Fig.7 DNA Fragmentation analysis**

Within limitations of our present study, it could be concluded that the extract of *Sida cordata* (burm.f.) Borss leaves exhibit a considerable anticancer activity against the Human tongue squamous cell carcinoma (CAL-27) cell lines and are nontoxic to normal human gingival fibroblast (HGF) cell lines

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