RESEARCH ARTICLE

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Evaluation of Therapeutic Potential of Eugenol-A Natural Derivative of *Syzygium aromaticum* on Cervical Cancer

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Abstract

Background: The intendment of this study is to determine the pursuance in – vitro anticancer activity and cytotoxicity of *Syzygium aromaticum* against the human cervical cancer cell line (HeLa) compared to the normal cell lines. Apoptogenic properties of DCM extract of Eugenol was determined in this entire study. **Materials and Methods:** HeLa cell lines were cultured in DMEM medium and incubated with different concentration of DCM – Eugenol extract. MTT assay brought out the way to determine the cell viability and quantification was done with the optical absorbance at 570 nm and 620 nm as reference. Apoptotic cells were affirmed by dual staining using acridine orange bromide. Besides, the morphology of the nucleus was also confirmed by dual staining. Eugenol inhibited 50% growth (IC50) of HeLa cell lines at 200 mg/ml of extract concentration. **Results:** Inhibitory efficacy of eugenol isolated from Syzyzgyium aromaticum showed the cell – viability in time and dose dependent manner with consistent morphological changes. Flow cytometer determined the apoptosis confirming the cytotoxicity value for MTT at IC50 with 81.85% cell viability. Dual staining firmly enacts the damaged cells due to AO indicating apoptosis confirmation by dual staining. Morphological analysis also clearly states that nil apoptosis has been seen in control and similarly in eugenol treated when compared to cancerous HeLa cell – line. **Conclusion:** Evaluation of cytotoxicity effect of eugenol isolated from *Syzygium aromaticum* showed it can be unrivalled dormant source of prodigious changes in HeLa cell line indicating (revealing) that chemotherapeutic agent.

Keywords: *Syzygium aromaticum*- HeLa Cell Line- MTT- HPLC- Eugenol- DCM

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Introduction

Cancer is one of the life threatening conditions caused by unbounded proliferation of cells. The enumeration of National cancer institute, USA, reports that there are more than 100 types of cancer and all cancers, however, fall into one of four broad categories such as Carcinoma, Leukemia, Sarcoma and Lymphoma. Carcinoma is developed from epithelial cells and this is the single largest group of human cancers forming about 80 percent of all cancers. Likewise, sarcomas are tumors developing in the connective tissue cells (mesenchymal tissue) such as fibroblasts or bone cells. Cancers of blood forming cells are called leukemia. Sometimes cancer produced from the lymphoid origin is localized in lymph glands called lymphomas (Liu et al., 2015).

Cervical cancer is a current malicious disease in women and the current reason of death (Wright et al., 2005; Colager et al., 2015) in women population. Developing countries are the victims for this disease when compared to advancing countries (DeVita and Chu, 2008). Cervical cancer is a heterogenous disease, since the causative agent is the genetic and environmental factors which level to the precocious clustering of epigenetic molecules in malignant cells (Walsh and King, 2007; Jemal et al., 2005). Various expression genes like CD44, E –cadherin KAI1, NH23, NME 1 are identified through various natural resources which are involved in the metastasis of malignant cells (Jemal et al., 2005).

Human papilloma virus (HPV) is the causative agent for cervical cancer and is the third common cancer diagnosticated in women (Zeng et al., 2014). There are about 200 types of HPV isolates prevalent in different zones in the world and it is reported that only 14 strains (1 6,18,31,45,33,35,39,51,52,56,58,66,68,70) accomplished the development of tumor in humans (Burd et al., 2003). The HPV oncoproteins are not the only factor that contributes to the progression of carcinoma but it enhances the response of cancer cells to estrogen (Megan et al., 2017). There are not many targeted therapies for treating cervical cancer. Hence development of effectual remedy is in demand today. It is reported that many plant derived

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chemicals are said to have anti-cancerous activity. These compounds strengthen the apoptotic property of the cells and control the division of the cell. Such phytochemicals are abundant in herbs and spices (Berrington and Lall, 2012), which are used in different cuisines of the world.

Clove (Syzygium aromaticum) is a flower bud used as a spice, for enriching the flavor of various foods. The flavor and aroma of the clove is due to the presence of eugenol (70-85%), an essential oil. The other two major compounds in the clove extract are Eugenyl acetate (15%) and β-caryophyllene (5-12%). It is proclaimed that eugenol is used as analgesics in dental treatment and is also combined with zinc oxide to be used as dental fillings (Sonalker and Nitave, 2014). They reveal anti-microbial against manyfoods borne pathogens (Singh et al., 2016). Additionally, it also exhibits strong anti-fungal property against Candida aspergillus and dermophyte species (Singh et al., 2016). Anti-oxidant property of eugenol is reported earlier with in vitro and in vivo studies (Nagababu et al., 2010). The current study pertains to the evaluation of cytotoxicity assay of eugenol against cervical carcinoma (HeLa cell lines) and in the furtherance of a fortune medication to relieve women from this battle for survival.

Materials and Methods

Extraction of eugenol fromSyzygiumaromaticum

Volatile components in clove were extracted using hydro distillation technique (Naveed et al., 2013). Steam distillate was prepared with 10 mg of powered clove using 80 ml of distilled water, and the volume was maintained by adding 20 ml of distilled water at equal intervals to prevent the deterioration of sample properties. The collected distillate was then subjected to liquid – liquid extraction by adding 15ml of dichloromethane and the final solute was dehydrated using anhydrous sodium sulphate (Rana et al., 2011). The residual solvent was evaporated by rotary evaporator in vacuum at 40^{o}C.

Identification and Purification of Bioactive compound
Phytochemical analysis of syzygium aromaticum extracts

The phytoconstituents present in DCM extract were determined quantitatively by HPTLC. The extract was dotted on silica coated plates and developed using butanol – glacial acetic acid-water (100:10:10). Vanillin solution (1%(W/V)) in 50% phosphoric acid was sprayed for steroic detection. Dragendorff's reagent and ethanoic benzidine was used to detect alkaloids and glucose concentration respectively (Ravi et al., 2013). The conformation of phytoconstituents were done with Keller – Killani test.

GC – MS analysis of bioactive components in DCM extract of Syzygiumaromaticum

Volatile nonionic and thermally stable components were analysed using gas chromatography – mass spectrometry (Sasidharan et al., 2011). Dried extract of Syzygiumaromaticum was dissolved in 95% v/v DCM and analysed using GC clares 500, perkinelmer USA equipped with turbo mass gold – perkinelmer detector. The column employed in integerated analysis was made of elite (5MS) [5% biphenyl 95%dimethypolysiloxane 30 m x 0.25 mm ID x 250 µm df] and carrier gas used to separate the components was helium with steady flowrate at 1ml/min. Temperature maintained was at 26oC for about 6 minutes and after 1µl of sample injection the temperature was increased by 10 ^{o}C min-1 with the initial range of 60 ^{o}C. Database search for the results was carried out by GCMS library (2008).

Structural prediction of phytoconstituents by FT – IR

The DCM extract was analysed by alternated total reflectance FTIR(ATR – FTIR) method using shimadzu at an infrared range of 400 – 4000 cm^{-1}. The sample was blended with KBr in the ratio of 1:9 and pelletized by 10 ton hydraulic pressure, and the results were analyzed at various absorption spectra.

HPLC analysis of DCM clove extract

The DCM extract (0.1g) was dissolved in 10ml of methanol. Membrane filter used was in the size of 0.2 cm (Millipore MA, USA). Column used was C18 – reversed phase silica bond clone column (3.9 x 300 mm). Peak ABC chromatography data handling system was used in processing chromatograph. Elution absorbance was measured at 280 nm. Triplication was carried out for all the injected volume of 20 µl. Reference standards were used to identify the obtained the retention time of unknown peaks to those of reference authentic standards.

Cell culture

Helacancer cells (HPV18+) were procured fromATCC and cultured in DMEM, supplemented with 2 mM glutamine, 10 µ/ml penicillin, 1.0 µ/ml streptomycin, 0.1 mM nonessential aminoacids and 1mM pyruvate sodium using 10% FBs. 5% CO_2 and 37 ^{o}C temperature was used to maintain the humid atmosphere. Medium was changed (passaged) at an interval of 3 days to ensure the viability of the cells.

MTT assay

Cytotoxicity and cellular viability of the cells were analysed by 3 – (4, 5 Dimethythiazol – 2yl) – 2, 5 diphenyltetrazonium bromide (MTT) assay. Different concentration (0.1, 0.2, 0.5, 1, 2, 4, 8 ong/ml) of eugenol was prepared and (HPV18+) Hela cell lines were treated at different intervals of time (24, 48 and 72 hours). After 72 hours the cell line was treatedwith MTT colour with a concentration of 0.5 mg/ml and kept at 37 ^{o}C at 5% CO_2 for 32 min. Later, DMSO was added to solubilise the formazan crystals and again followed by a incubation period of 30 min at room temperature. Soon after the incubation time, the samples were measured at a wavelength of 540 nm (Mosmann, 1983) using ELISA reader (oragcnon ELISA reader teknika, Netherland) viability of the cells was confirmed as follows:

Viability = ((Absorption of treated samples x 100) / Absorption of controls)

This method was followed to find the ratio of half maximal inhibitory of positive and negative controls

(Rafieian et al., 2014).

Dual staining / AO/EB Fluorescent staining

Apoptosis analysed using fluroscent problems of acridine orange and ethidium bromide(Lakshmi et al., 2008). After cytotoxicity treatment medium was removed from the plates, cells of (1x 10^5) were washed with 1 x PBS. Fluroscent microscopy was used to interpret the results and the morphological report was photographed.

Western blot analysis

HeLa (HPV 18+) cells were grown in a 10 cm dish. When cell density reached 80–90% confluence, cells were treated with Eugenol (500 µg/ml) for the indicated times. The cells were then washed once with icecold PBS and lysed with lysis buffer (20% SDS containing 2 mM phenymethylsulfonyl fluoride, 10 mM iodoacetamide, 1 mM leupeptin, 1 mM antipain, 0.1 mM sodium orthovanadate and 5 mM sodium fluoride) for 10–20 min. The lysates were sonicated 3× at 10 sec intervals, aliquoted and stored at −20°C. The protein concentration was determined by the BioRad DC protein assay (BioRad Laboratories, Hercules, CA). Equal amounts of protein (20 µg/lane) were subjected to 12% SDS-PAGE and transferred to a nitrocellulose membrane. The membranes were subsequently incubated with the corresponding primary antibodies, as indicated: a rabbit antiBeta 2 polyclonal antibody, a mouse antiBcl_2 monoclonal antibody (Sigma);, a rabbit anticaspase3 polyclonal antibody (Sigma Aldrich). Antibody recognition was detected with the respective secondary antibody, ether antimouse IgG, or antirabbit IgG antibodies linked to horseradish peroxidase (Zymed). Antibody bound proteins were detected by the ECL western blotting analysis system.

Results

Phytochemical analysis

Prefatory screening of SyzygiumaromaticumDCM extracts showed the presence of diversity of phytochemical constituents. The presence of active secondary metabolites was confirmed by the election of alkaloid, steroid, flavanoid and terpenoid according to the procedure followed by Keller – Killani test and the results are established in Table1; Figure 1.

GC-MS analysis of bioactive components in DCM extract of Syzgium aromaticum

Syzygiumaromaticum were found to contain many aliphatic compounds like eugenol, caryophyllene, alpha caryophyllene. Eugenol is one of the aromatic

Table 1. Preliminary Phytochemical Screening of *Syzygium Aromaticum* Extract

Test For Extract	Inference
Alkaloid	+
Flavanoid	+
Steroid	+
Terpenoid	+

+, Presence; -, Absence

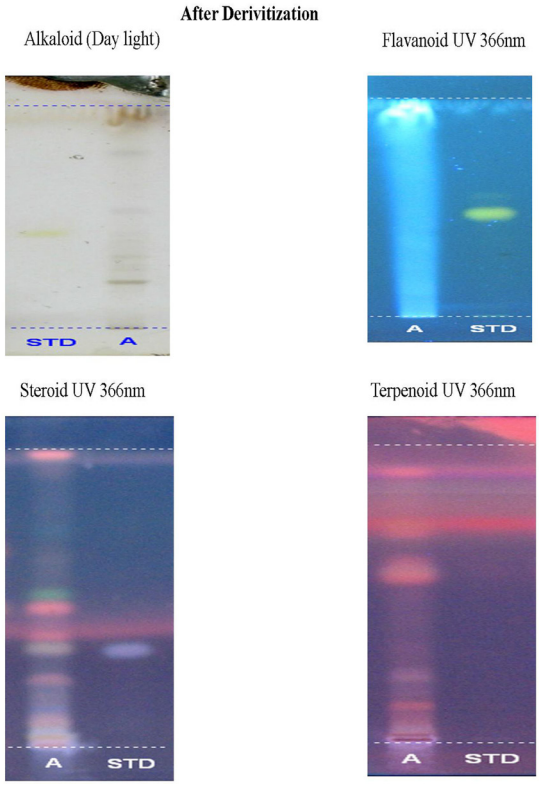


Figure 1. TLC Plates Showing the Presence of Alkaloid, Flavanoid, Steroid and Terpenoid after Derivitization Process

compoundsand that lead to the pleasant aroma of the Syzygiumaromaticum DCM extract. Besides GCMS results also proved that eugenol is one the main phytoconstituents in the extract. The peak obtained was analysed with the help of NIST library (200s) and the results are indicated in Table 2; Figure2.

FT-IR

FT-IR remains the most robust tool for identifying the types of chemical bonds (functional group) in compounds present ina sample. Annoted spectrum denotes the characterisation of chemical bond detected by the light absorbed at each wavelength. The DCM extract showed the characterisation absorption bands at

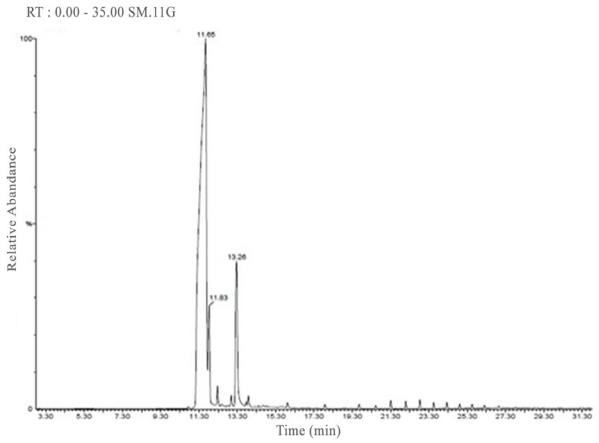
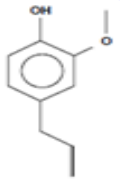
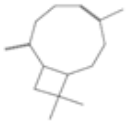
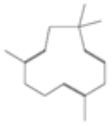


Figure 2. Chromatogram (GC-MS) of DCM Extract of Syzygium Aromaticum

Table 2. Structural Prediction of Bioactive Components from GC-MS Analysis of *Syzygium Aromaticum* Extract

Retention time	Compound name	Molecular formula	Molecular weight	Area%	Structure
11.657	Eugenol	C10H12O2	164	83.785	
11.847	Caryophyllene	C15H24	204	5.191	
13.268	Alpha Caryophyllene	C15H24	204	11.024	

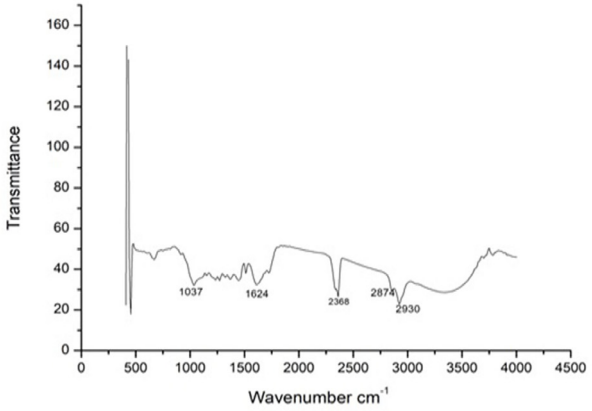


Figure 3. Infrared Spectrum (FT-IR) of DCM Extract of *Syzygium Aromaticum*

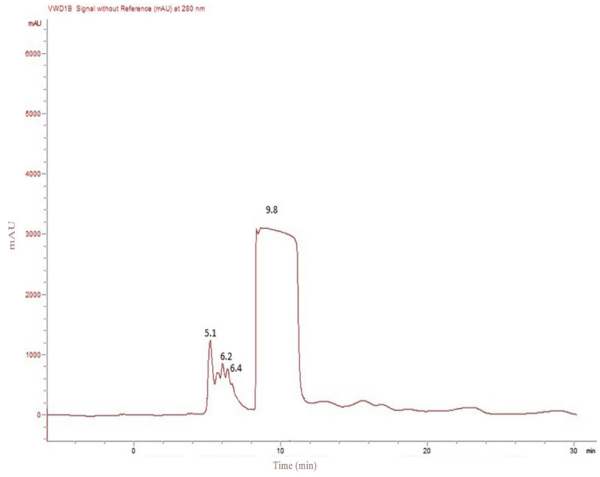


Figure 4. Chromatogram (HPLC) of Eugenol Standard

400 Cm^{-1} to 4,000 Cm^{-1} in Figure 3, out of this bonds absorption spectra from 292 Cm to 2,950 Cm for (C-H) stretching, 1,377 Cm^{-1} and 1,450 Cm^{-1} (for C-H bonding) for C-H group at 1,701Cm^{-1} for carbonyl group (C=0). The band at 1,064Cm^{-1} is due to OH group (Table 3).

HPLC

HPLC is a ambidextrous prominent and universally used technique for the desolution of natural products from a extract. In this the DCM extract of Syzygiumaromaticum was undergone extraction by areverse phase silence bond technique. Various bioassay aided fraction elected was collected separately and qualitative analysis was performed for the eminent identification of eugenol. The eluted sample was confirmed as eugenol was again confirmed through HPLC by running against standard as shown in Figure 4 and Figure 5.

Effect of eugenol on HeLa (HPV 18) cervical cancer cell line

MTT assay performed using Hela cell line revealed that eugenol decrease the viability of cells Inducing

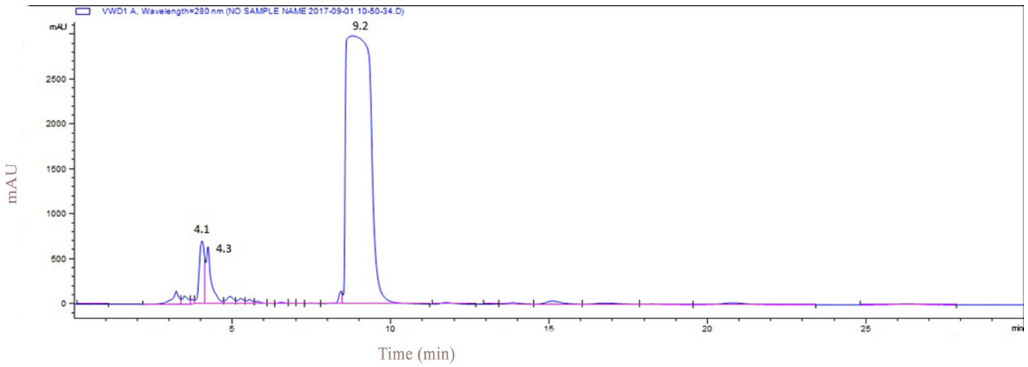


Figure 5. Chromatogram (HPLC) of Eugenol Separated from Crude DCM Extract of *Syzygium Aromaticum*

Table 3. Different Functional Groups of *Syzygium Aromaticum* Respect to Their Wavenumbers Obtained by FT-IR

S.NO	Wave Number (cm^{-1})	Functional Group
1	1037	C-O (Alcohols,Ethers,Esters,Carboxylic acids,anhydrides)
2	1624	C=C (Alkene); C=O (Amide)
3	2368	No corresponding functional group were noticed.
4	2874	C-H (Alkanes (stretch), Aldehyde)
5	2930	O-H (Carboxylic acids); C-H (Alkanes (stretch))

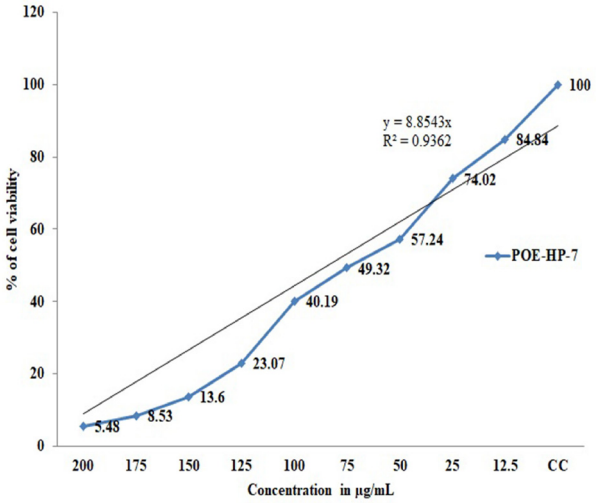


Figure 6. *In Vitro* Cytotoxicity Effect of Eugenol Against HeLa Cell Line

cytotoxicity of cells. IC50 was confirmed according to the cytotoxicity studies for a time period of dose for the impending tests indicated in Table 4. Cytotoxicity studies for a time period of 4, 8, 16 and 24 hour assayed, Eugenol extract of Syzgiumaromaticum displayed cytotoxic effect in dose dependent manner, with the highest concentration of 1mg ml-1, the eugenol extract inhibit the growth of Hela cancer cells and provided a prominent cytotoxicity effect to the cell lines in a concentration (0.05, 0.1, 0.5, 0.75, 1 mg m-1) were decreased. Concentration of 1 mg ml-1 in allotted time period of 4, 8, 16, 24 hour cells had 57%, 33%, 20%, 18% as shown in Figure 6 and Figure 7.

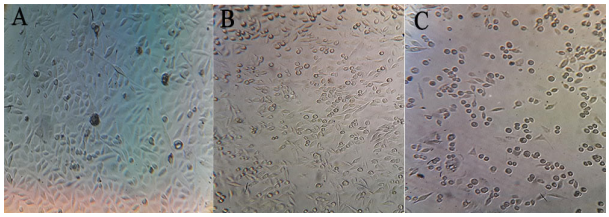


Figure 7. 7A) Negative Control Group: untreated cells exhibiting normal shape with clear outline; 7B) Experimental Group: Hindered cell growth, proliferation was inhibited and slowed; 7C) Necrotic Group: Compute inhibition of proliferation and growth.

Table 4. *In Vitro* Cytotoxicity Effect of Eugenol Against HeLa Cell Line

Sample Conc. (mg/mL)	%Cell Viability
0	100
12.5	84.84
25	74.02
50	57.24
75	49.32
100	40.19
125	23.07
150	13.6
175	8.53
200	5.48
IC50	81.85

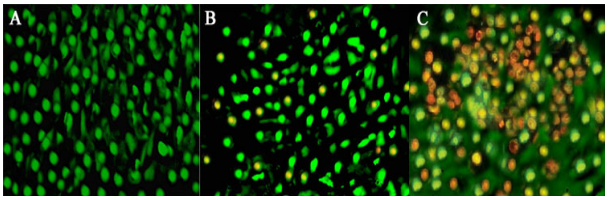


Figure 8. 8A) Negative control (normal cells): The circular nucleus uniformly distributed in the centre of the cell; 8B) Experimental Group (early apoptotic cells): nucleus showed yellow - green fluorescence by acridine orange (AO) staining and concentrated into a crescent or granular that located in 1 side of cells; 8C) Necrotic Cells: The necrosis cell's volume was increased, showing uneven orange – red fluorescence and an unapparent outline. It is becoming dissolved or near disintegration.

Dual staining

Morphological study of the cell shape was performed by acridine orange and ethidium bromide staining. Using an inverted phase contrast microscope (400x) revealed that the treated cells with 0.5 and 1 mg ml-1 after 24 hours exhibited typical characteristics of apo – neurosis indicating nuclear condensation indicated in dark blue colour. The normal cells showed normal nuclei, homogenous and dark blue. Thus it was revealed that eugenol extract treated cells were round, Proliferation was inhibited and showed in Figure 8.

Cervical cancer cells were labelled by AO/EB 24hours

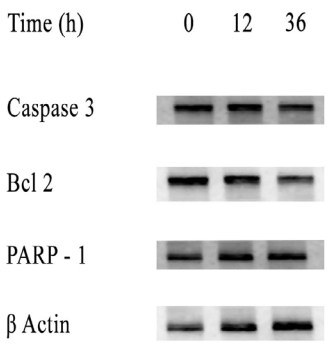


Figure 9. Effect of Eugenol from *Syzygium aromaticum* on expression of caspase-3 and PARP in HeLa cells.

after eugenol was applied. Dual staining was conducted under a fluorescent microscope. Negative control showed no apoptosis as shown in (Figure 8A). Early apoptosis, marked by granular yellow – green AO nuclear staining, are shown in experiment group (Figure 8B). Localization of stain was carried out asymmetrically within cells. With increasing concentration such as IC80 showed uneven orange – red fluroscence at their periphery affirming the disintegration of apoptic cells as shown in (Figure 8C).

Western Blotting Analysis

The potential signaling pathways underlying Syzygium aromaticum–Eugenol extractinduced apoptosis was evaluated using Bcl2 family proteins by Western blot. The Bax protein level was increased 12 hr after treatment and remained elevated up to 48 hr. No change was observed in protein expression of Bcl2. Assessment of caspase3 in this apoptotic process was carried out by evaluating the expression of caspase3. As shown in Figure 9 ,the 35 kD proenzyme caspase3 was cleaved to its active 20 kD form 12 hr after treatment in a time dependent manner. The 116 kD PARP protein was cleaved to 85 kD fragment in a time dependent manner to a maximum level at 36 hr.

Discussion

In this research, the DCM extract of *Syzygium aromaticum* was analyzed for apoptosis of HeLa cancer cells. The extract was analyzed through GC-MS, where three compounds showed large area with strong peaks. Out of three, eugenol was immaculated using HPLC for further resolution.

This investigation clearly illustrates the existence of eugenol in DCM extract of *S. aromaticum*, which was affirmed by GC-MS analysis of biological sample and the structure of the compounds are also predicted by FTIR technique. GC-MS is regarded as the robust technology to anticipate the existence of discrete compounds in the plant extract. Whereas, further studies similar to this gives a clear cut view of omnipresence of eugenol in wide range of essential oil constituents. A common spice that involve eugenol was cinnamon bark which contain many additional compounds in spite of eugenol (Adinew, 2014). There are other several methods to validate the existence of eugenol, however this study involves hydro distillation process to cull clove oil from the provided sample (Athar et al., 2013). The diversity in the ring structures of the chemical groups were evaluated in this study and also methyl eugenol was seen in disparate (Raymond et al., 2017). The biosynthesis of these bioactive compounds and also their systematization in sub cellular level was visualized in wide categories of plant species. Since these compounds are found to have a broad range of applications in various fields dawn from ointments to drugs that can cure chronic diseases (Kaul et al., 2008). From the above results it is commenced that retention time of eugenol was found to be 11.657 mins whereas methyl eugenol was 16.77 mins.

Initially, the extract was analyzed through High Performance liquid chromatography to resolve the different peaks. The dichloromethane extract was used to determine the existence of eugenol by comparing various traits of the sample with the standard. However, the ethanolic extract of clove cater the retention time of about 20.4 mins in analogy with the standard chromatogram (Tsai et al., 2017). Analyzing the sample through HPLC, UV detectors play a vital role for attaining the desired peak, which can be observed at 280 nm. Of all the process of dealing with the crude extracts, HPLC was viewed as the convenient choice for examining the natural derived products. The versatile nature of HPLC accord the well-presented chromatogram for secondary metabolites in the plants. Hence, it affords distinct applications in isolation and quantification of the bioactive components from various sources in the nature. Addition to that, reverse-phase column contributes for the enhanced separation and detection of components from the wide range of mixtures due to its selectivity and robustness (Boligon and Athayde, 2014).

The procured cytotoxicity results provide a clear cut view of effect of eugenol on cancer cell lines. Analysis of this study illustrates that eugenol can act as effective agent contrary to cancer cell line such as HeLa than other chemotherapeutic agent commercially available in the market. Eugenol not only showed its actions in HeLa cells but also in other cell lines likes lung cancer and breast cancer. Globally, cancer is advertised as the deadly disease from which numerous people lost their lives by diverse type of cancer originating from distinct parts of their body. Accordingly, numerous research is enduring for better evolvement of medicines treating against cancer (Greenwell and Rahman, 2015). Eugenol is said to have a defense action by which the compound knocks off the expression levels of phosphate-Akt and MMP-2 in lung cancer cells (Fangjun and Zhijia, 2018). In breast cancer cell lines like MCF-7, MDA-MB 231 and 4 T1, the extract of Annona muricata exhibited better anti-cancer activity (Levitsky and Dembitsky, 2015; Faruq et al., 2016) Many naturally available compounds from plants are proclaimed to contain anti-cancer, anti-inflammatory and anti-oxidant activities. Plant derived compounds are reported to enhance the availability of drug in the intestines, which is used to forbid the surge of cancer cells (Liu et al., 2014). Some of the pigments in plants act as natural prevention against cancer and raise their drug ability to the site of cancer. The various anti-tumour phyto pigments are Apigenin, Curcumin, Cyanidins, Indole-3-carbinol (I3C), Fisetin, Gingerol etc (Wang et al., 2012). Eugenol loaded nano emulsions induces apoptosis therefore both cell cycle and apoptosis was resolved by flow cytometry. Liver cells (HB8065) and Colon cells (HTB37) promotes greater apoptosis percentage when its treated with eugenol and canola oil solution. Morphology of treated cell lines can be visualized using converted fluorescent microscopy by staining with Hochest33258 (Majeed et al., 2014). Down regulation of E2F1 by intrinsic pathway of apoptosis which was regarded as the main killing effect of tumours. Existence of eugenol in the medium enhance the up-regulation of cyclin-dependent kinase inhibitor p21WAF1 protein which would arrest the uncontrolled growth of breast cancer (Sharif et al., 2013). Effect of eugenol in breast cancer cells depends on the

range of concentrations which was found to promote the cell death and also relay on dosage and time of exposure (Vidhya and Devraj, 2011).

Urtica membranacea shows improved activity than other plant extracts (Solowey et al., 2014). One of the research study shows that the activity of prostate cancer cell exhibits suppression when treated with Leea indica leaf extracts on different cell lines like DU-145 and PC-3. The cytotoxicity effects of methanol and ethanol extracts possess selective cause in prostate cell lines (Ghagane et al., 2017). Omnipresence of eugenol acts contrary to colon cancer cells by endorsing apoptosis in HCT-15 and HCT-29. This can be determined by measuring MMP and different order of ROS generation in cancer cells after certain limit of treatment (Jaganathan et al., 2011). Our study mainly focuses on vivacity of naturally existing drug named eugenolcontrary on HeLa cell line, which could enhance the apoptosis of abnormal cells in the body. A plant species called Ficus tend to cytotoxic human carcinoma cells especially HeLa cells. The study on this plant reveals that the extract of this plant promotes cytotoxicity even at low concentrations of about 2µg/ml (Khodarami et al., 2011). A comparative study was done with Papaver alkaloids on normal (Vero) and cancerous cell line (Artun et al., 2016) and it served as a effective drug against Helacells. Bryonia aspera extract promotes better cytotoxicity and apoptogenic mechanism against HeLa and HN-5 cell lines (Demirgan et al., 2016). The morphological changes in these cell lines occur due to inhibitory actions which was observed by light microscope (Pourgonabadi et al., 2017). Methanol extract of Pseudocedrela kotschyi exhibits better action towards HeLa, MCF-7, and RD cell lines. The obtained results are compared with the commercially available drugs such as vinblastine and methotrexate and is suggested that extracted sample exhibit better action than the already existing drugs (Elufioye et al., 2017). Non-pathogenic bacteria which significantly involve in fermentation process belongs to Lactobacillus species and is regarded as the effective contrary to cancer. One among those species named Lactobacillus Casei which is used as a starter culture for fermented products can be developed into efficient drug to treat cervical cancer. Foods enriched with such products are essential for women to fight against this deadly defect, caused by the psychotic changes in the life style (Kim et al., 2015).

Combination of myricetin and methyl eugenol also boost the anticancer activity, cell cycle arrest and it also promotes action of cis-platin on other hand which induces apoptosis of cervical cancer cells (Yi et al., 2015). Expression of Caspase and BID activity on HeLa cells were seen after treating cells with Kaempferia parviflora extract to examine the phosphorylation status of various genes such as ERK1/2, Elk1, AKT, and PI3K. The expression levels observed through these genes showed the enhanced level of apoptotic protein which was detected using effective chemiluminescence reagent such as horseradish peroxidase (HRP) and then the bands were evaluated using the particular software (Potikanond et al., 2017). The anti-tumor property of Kelussia odoratissima and Silene viscidula extractswere seen and investigated through MTT assay. Cell cycle analysis was carried out by flow cytometry and sinocrassulosides VI/VII from Silene viscidula were evaluated contrary to human colorectal cancer cell lines SW620 and HT-29, the human gastric cancer cell lines SGC-7901 and BGC823, the human lung cancer cell line A549, the human glioblastoma cancer cell line U87MG, the human breast cancer cell lines MCF-7 and MDA-MB-435and the human cervical cancer cell line HeLa. Therefore, their inhibitory effects promoted by compounds which was observed through MTT assay (Hosseini et al., 2017). Our results for the first time demonstrate that the Eugenol is directly capable of inducing apoptosis in human breast cancer cells, not through immune system. Our results further show that this apoptotic process might be mediated through up-regulation of Poly (ADP-ribose) polymerase (PARP), PARP is cleaved by caspase-3 early during apoptosis in many different cell lines. The cleavage of PARP between Asp214 and Gly215 results in the separation of the two zinc-finger DNA-binding motifs in the NH2-terminal region of the enzyme from the automodification and catalytic domains, thus preventing the recruitment of the catalytic domain to sites of DNA damage (Berger, 1985). This cleavage of PARP has been suggested to occur in order to prevent depletion of energy (NAD and ATP) that is thought to be required for later stages of apoptosis. PARP cleavage serves to prevent futile repair of DNA strand breaks during the apoptotic program. a member of Bcl2 family. The proapoptotic Bax protein led to mitochondrial dysfunction and the release of cytochrome c (Cyt c) from the mitochondria. The released Cyt c then interacts with specific adapter, such as Apaf1, which in turn proteolytically converts procaspases to active caspases. One key step of this cascade in HeLa cells was activation of caspase7, which cleaves several substrates including the PARP, a nuclear enzyme involved in DNA repair and maintenance of genome integrity and posttranslational ribosylation of proteins, whereby apoptosis occurs. The inactive caspase3 precursor was cleaved to the 20 kD subunit forming the active protease during apoptosis (Germain et al., 1999). This occurred in conjunction with cleavage of 116 kD PARP to 85kD proteolytic fragments. Results suggest that caspase3 may be the main effector caspase in caspase3 deficient MCF7 cells during apoptosis (Hu et al., 2001).

From this investigation, it is concluded that the activity of bioactive compounds showing a contrary effect against HeLa cancer cell lines was observed. The MTT assay was carried out with eugenol against cervical cancer cell lines and it illustrate the cell viability with respect to the natural drug and provide us a better IC50 value for contrary to cancer cell line. After incubation of treated cells with distinct concentrations of the desired phytocompound for 24 hrs, it was observed for apoptosis of HeLa cells through fluorescence microscopy by staining with acridine orange and ethidium bromide. This provides us a clear view in the increased rate of apoptosis compared to other commercially available drugs (Liu et al., 2001).

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