WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 15, 1097-1114. Research Article ISSN 2277– 7105

# INVITRO STUDIES ON THE EFFECT OF *ZINGIBER OFFICINALE* METHANOL EXTRACT IN LUNG CANCER A549 CELL LINES

**[1]Rejilal R. Nair, [2]Dinesh Bhaskar, [3]Khalidah Khalaf Jabbar, [4]Nagarathna Amresh, *[5]Dr. Balasubramanian Sathyamurthy**

[1,2]Department of Biochemistry, REVA Institute of Science and Management, Bangalore – 560064.

[3]Department of Biochemistry, Acharya Nagarjuna University, Guntur, Andhra Pradesh, India.

[4]Principal, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.

[5]Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.

Article Received on
30 Sep. 2017,

Revised on 20 Oct. 2017,
Accepted on 10 Nov. 2017

DOI: 10.20959/wjpr201715-10174

***Corresponding Author**
**Dr. Balasubramanian Sathyamurthy**
Professor, Department of Biochemistry, Ramaiah College of Arts, Science and Commerce, Bangalore – 560054.

## ABSTRACT

The root of *Zingiberofficinale* is regarded as Ginger plant and commonly used in traditional medicine by many Countries like India, China, Nigeria, Indonesia, Bangladesh, Thailand, Philippines, Jamaica etc. to treat anticarcinogenic activity, antioxidant, anti inflammatory activityand many other diseases. This root extracts are found to have antioxidant and anticancer activities. Gingerol is the active principle which is responsible for these beneficial effects. This work was aimed to study the effect of *Zingiberofficinale* methanolic extracts on A549 lungs cancer cell lines and found these methanol extracts has high phytochemical compounds having more free radical scavenging and anticancer activities.

**KEYWORDS:** *Zingiberofficinale*, Gingerol, Flowcytometer, ginger, A549 cell line

## INTRODUCTION

In the last century, significant development in bio-medical science has conquered many diseases however cancer remains ambiguous especially from a therapeutic perspective. Cancer is still a growing health problem world-wide, and it is the second most common cause of death from disease after myocardial infarction. Tumor or neoplasm is usually defined as a

growth of an abnormal mass of tissue due to uncontrolled cellular growth, while cancer is the term of all malignant tumors. There are thousands of scientific studies that have focused on the pharmacological activity of bio-active components from plants, increasing interest from scientific community as cancers suppressant. Biological targets of phytochemicals in mammalian cells were found to be involved in inflammatory of cell cycle control, apoptosis evasion, angiogenesis and metastases. Additionally, epidemiological studies suggest that the daily intake of certain photochemical can reduce the incidence of several types of cancers.[1]

Lungs being an important organ in the respiratory system include nose, mouth, windpipe (trachea) and airways. The airways to each lung are called large airways (bronchi) and small airways (bronchioles). Lung cancer is the second most common cancer in both men and women and the leading cause of cancer death among both men and women in the world. It accounts for about 14% of all new cancers in both men and women. For smokers the risk of lung cancer is much higher than in non-smokers. There are 2 major types of lung cancer, Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC). 85 - 90% of all lung cancers are NSCLC. Uranium is the main cancer causing agent which is found in rocks and soil. As it decays, a gas called radon is made and gets into air and water. Miners of uranium have a high risk of developing lung cancer. The risk for lung cancer may depend on how much radon gets emitted. Besides radon, there are 10 other agents known to cause lung cancer. Five are metallic metals: arsenic, beryllium, cadmium, chromium, and nickel. The others are asbestos, coal smoke, soot, silica, and diesel fumes. Among people who've had major contact with these agents, the risk for lung cancer is higher for those who are having the smoking habit. [2]

**A549 cell line**

| | | |
|---|---|---|
| **Organism** | **:** | *Homo sapiens*, human |
| **Tissue** | **:** | Lungs |
| **Disease** | **:** | Carcinoma |
| **Age** | **:** | 58years |
| **Gender** | **:** | Male |
| **Morphology** | **:** | Epithelial |
| **Growth Properties** | **:** | Adherent |

The A549 cell line (ATCC: CCL-185) was established by D.J. Giard, et al. (Giard et al., 1973) in 1972. The cells originate from an explants culture of lung carcinoma tissue from a

58-year-old Caucasian male. This was an attempt to establish continuous cell lines from 200 different tumours. This A549 Cell line is being developed as a tool for cancer research the cell line was consequently characterized as being represents of the Alveolar Type II pneumocytes of the human lung and because of this the cell line has been a main stay of respiratory research for nearly forty years. A549 cell line has the ability to exhibit features of an ATII epithelial cell phenotype having expression of high numbers of Multilamellar bodies (MLB) indicating the cell might be capable of surfactant production. More contemporary studies led to a more conservative view on the cell line's suitability to recapitulate the ATII phenotype.[3]

Nearly 80% of lung cancers are adenocarcinoma, which usually originates in peripheral lung tissue. Adenocarcinoma i**s** a type of cancerous tumor that can occur in several parts of the body. It is defined *as* neoplasia of epithelial tissue that has glandular origin, glandular characteristics, or both. Adenocarcinom as are part of the larger grouping of carcinomas. The main application of this cell lines is the formation of epithelial models of the distal lung which is used for studying the airway function, viral diseases and in cancer research. The A549 cell line is a suitable host for many human respiratory viruses including adenoviruses. A549 cells grow as an adherent monolayer and can be used as a transfection host.[4]

***Zingiberofficinale***

Ginger scientifically known as *Zingiberofficinale Roscoe*, belonging to family *Zingiberaceaeis* is one of the most important plant with several medicinal and nutritional values. It is used extensively worldwide as a spice, flavoring agent and herbal remedy. Traditionally, *Z. officinale* is used in Ayurveda, Siddha, Chinese, Arabian, Africans, Caribbean and many other medicinal systems to cure variety of diseases like nausea, vomiting, asthma, cough, palpitaion, inflammation, dyspepsia, loss of appetite, constipation, indigestion and pain. *Zingiberaceae* family is the largest family of *Zingiberales* and one of the ten largest monocotyledonous families in India. It occurs chiefly in the tropics with about 52 genera and 1400 species. In India there are about 22 genera and 178 species. *Zingiberaceae* forms an important group with economic potential and many members of this family yield spices, dyes, perfumes and medicines and some are ornamental.[5]

*Zingiberofficinale Roscoe* has several biological properties like antimicrobial, antioxidant, anticancer, that stimulate effect on the immune system. *Zingiberaceae* extracts containing many essential oils like terpenes, alcohols, ketones, flavanoids, carotenoids, gingeroles, and

phytoestrogens. Ginger is supposed to have originated in South-East Asia. Currently, India and China are the dominant suppliers to the world market. Ginger is grown in many countries like India, China, Nigeria, Indonesia, Bangladesh, Thailand, Philippines, Jamaica, Australia, Brazil and Japan. Nigeria ranks first with respect to area under ginger covering about 56.23% of the total global area followed by India (23.6%), China (4.47%), Indonesia (3.37%) and Bangladesh (2.32%). In production India stands first contributing about 33% of the world's production followed by China, Nigeria and Bangladesh. Japan and USA are the major importers. Australia is the world leader in value added products. India has 50% share in oil and oleoresin trade. In India, ginger is cultivated in Kerala, Meghalaya, Arunachal Pradesh, Mizoram, Sikkim, Nagaland and Orissa altogether contributes 70% to the country's total production. In terms of quality, Jamaican and Indian ginger are considered to be superior followed by West African variety.[6]

Ginger plant is herbaceous and grows up to95 cm in height under cultivation. Rhizomes are aromatic, thick lobed, pale yellowish, bearing simple alternate distichous narrow oblong lanceolate leaves. The herb forms several lateral shoots in the form of clumps, which begin to dry when the plant matures. Leaves grow long with 2 - 3 cm broad with sheathing bases, the blade gradually tapering to a point. Inflorescence is solitary, lateral radical pedunculate, oblong cylindrical spikes. Usually Flowers are rarely found rather small in size calyx is superior, gamosepalous, three toothed, open splitting on one side, corolla of three sub equal oblong to lanceolate connate greenish segments.[7]

**Chemical composition**

Phytochemical studies show that ginger rhizome contains wide variety of biologically active compounds which impart medicinal property. *Z. officinale* is reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups. The chemistry of *Z. officinale* has been the subject of sporadic study since the early 19$^{th}$ century. In common with some other pungent spices, considerable advances were made in the early part of the 20$^{th}$ century, but it has only been in recent years that a fairly clear understanding of the relationship of its chemical composition to its organoleptic properties has emerged. Ginger owes its characteristic organoleptic properties to two classes of constituents: the odor and much of the flavor of ginger is determined by the constituents of its steam-volatile oil, while the pungency is produced by non steam- volatile components. The aroma and flavor of ginger

are determined by the composition of its team volatile oil, which is comprised mainly of sesquiterpene hydrocarbons, monoterpene hydrocarbons and oxygenated monoterpenes. The monoterpene constituents are believed to be the most important contributors to the aroma of ginger and they tend to be relatively more abundant in the natural oil of the fresh ('green') rhizome than in the essential oil distilled from dried ginger. Oxygenated sesquiterpenes are relatively minor constituents of the volatile oil but appear to be significant contributors to its flavor properties. The volatile oil consists mainly of the mono- and sesquiterpenes;camphene, *β*-phellandrene, curcumene, cineole, geranylacetate, terphineol, terpenes, borneol, geraniol, limonene, *β*-elemene, zingiberol, linalool, *α*-zingiberene, *β*-sesquiphellandrene, *β*-bisabolene, zingiberenol and *α*-farmesene. Zingiberol is the principal aroma contributing component of ginger rhizome.[8]

Ginger contains biologically active elements such as the gingerols, shogaols, paradols and zingerone. The gingerols, were identified as the important active components in the fresh rhizome, it is a chemical homologs differentiated by the length of their unbranched alkyl chains. The spicy of dry ginger mainly results from shogaols, which are dehydrated forms of gingerols. Gingerols readily undergo dehydration to form the corresponding shogaols. Paradol is same to gingerol and is formed on hydrogenation of shogoal. Oleoresin obtained by acetone and ethanol extraction, contains 4 – 7.5% of dried powder.[9]

**Role of Gingerols**

Ginger root and its main phenolic compounds such as gingerols and zerumbone have anticarcinogenic activity, antioxidant and anti inflammatory activity. Specially, the constituents of ginger root can inhibition of activation of NF-kB induced by a variety of various factors. Ginger rich with many active components. The [6]-gingerol, a major pungent ingredient of ginger is a potent anti-angiogenic activity in vitro and in vivo. [6]-gingerol may inhibit tumor growth and metastasis via its anti-angiogenic activity. Topical application of [6]-gingerol inhibited COX-2 (cyclooxygenase-2) expression along with suppressed NF- kB DNA binding activity in mouse skin.[10]

The mechanisms involved in anticancer and chemopreventive properties via multiple pathways include inhibition of CycloOxygenase -2 (COX-2) expressions by inhibiting p38 MAPK–NF-κB (mitogen activated protein kinase – necrosis factor kappa B) signalling pathway. Ginger is a natural antioxidant and anticarcinogenic dietary component. The *invitro* treatment with ginger on certain cancer cells reported that 6 – Shogaol inhibits the growth of

cells by inhibition of NF- kB activation and decreases VEGF (growth factor) and IL-8 secretion. Ginger components modulate secretion of angiogenic factors in ovarian cancer cells *invitro* and act as potent chemopreventive dietary agent.[11]

The β- elemene which is known as a novel anticancer drug is extracted from the ginger plant and triggers apoptosis mediated through a mitochondrial release of the cytochrome c in non-small-cell lung cancer cells. The β-elemene induces caspase3, 7 and 9 activities, decreases Bcl-2 expression, causes cytochrome c release and increases the levels of cleaved caspase9 and poly (ADP-ribose) polymerase in cells. Enhanced enzyme activity of Glutathione Reductase (GR), Glutathione Peroxidase (GPX), Glutathione – S – Transferase (GST) leads to the suppression of colon carcinogenesis by ginger supplement. Ginger effectively reduces the colon cancer.[12]

*Invivo* studies with ginger and its component [6] – gingerol shows effective against ovarian, skin, breast and colon cancers. Ginger inhibits NF-kB and also interleukin- 8 (IL-8) inhibitions. [6] – gingerol and [6] shogals inhibits gastric cancer. The ginger constituents including [6] – shogaol, [6] – gingerol, [8] – gingerol and [10]-gingerol were examined in humans to study pharmacokinetic properties of anticancer agents. Treatment with ginger extracts in hepatic caricoma cells of rats has reduced the elevated expression of tumor necrosis factor - alfa (TNF– α) and NF-κB. The intake of ginger reduced lipid peroxidation and acts as an antioxidant by which suppresses liver carcinogenesis. There are three ginger compounds include [6]-, [8]-, [10] - Shagaols are much stronger against tumor growth, observed in H-1299 human lung cancer cells and among these three [6] - Shagaol shows potential agent than [6] – gingerol.[13]

**Cell Cycle Analysis**

Reproduction of cells requires cell division, with production of two daughter cells. The most obvious cellular structure that requires duplication and division into daughter cells is the cell nucleus - the repository of the cell's genetic material, DNA. With few exceptions each cell in an organism contains the same amount of DNA and the same complement of chromosomes. Thus, cells must duplicate their allotment of DNA prior to division so that each daughter will receive the same DNA content as the parent. The cycle of increase in components (growth) and division, followed by growth and division of these daughter cells, etc., is called the cell cycle. The two most obvious features of the cell cycle are the synthesis and duplication of nuclear DNA before division, and the process of cellular division itself -mitosis. These two

components of the cell cycle are usually indicated in shorthand as the "S phase" and "mitosis" or "M" .When the S phase and M phase of the cell cycle were originally described, it was observed that there was a temporal delay or gap between mitosis and the onset of DNA synthesis, and another gap between the completion of DNA synthesis and the onset of mitosis. These gaps were termed G1 and G2, respectively. The cycle of G1 → S → G2 → M → G1, etc., is shown schematically in Figure 1.[14]

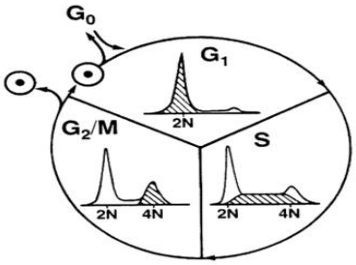


**Figure – 1: A cell cycle, showing flow cytometric components of each phase.**

## MATERIALS AND METHODS

### Sample Collection

*Zingiberofficinale* leaves and root were collected from Department of Horticulture, Gandhi Krushi Vignana Kendra (GKVK), Bangalore.

### Extraction procedure

Plant leaves and roots were washed thoroughly with distilled water and dried under shade at room temperature. The dried roots of *Zingiberofficinale* were finely grinded using electrical grinder and stored in air tight containers for further use. A total of 250 g of the pulverized plant material was extracted using methanol. The extracts were then filtered through Whatman's No. 1 filter paper and condensed to dryness using rotary evaporator. The thick extracted mass was dried at room temperature and used for the analysis.[15]

### 1. Phytochemical analysis

Phytochemical analysis of *Zingiberofficinale* root extracts were done using the protocols described by Segelman AB, Fransworth NR, Quimbi MD, L Loide for the following.[16]

Test for Sterols - Liebermann Burchard reaction
Tests for Alkaloids - Mayer's and Wagner's test
Tests for Tannins - Ferric chloride reagent test and
Tests for Saponins - Foam test
Tests for Phenols - Ferric chloride reagent test

Tests for Flavonoids - Sodium hydroxide test

Test for Terpenoids - Salkowski test

**2. Nitric oxide radical scavenging assay**

**Principle**

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be measured using Griess reagent at 546 nm spectrophotometrically.

**Procedure**

Nitric oxide scavenging assay is carried out as per the method of Sreejayan and Rao *et al*. In brief, 200 µl of 10 mM sodium nitroprusside and 200 µl of test solution/reference standard of various concentrations are incubated at room temperature for 150 minutes. Add 500 µl Griess reagent and incubated for 10 min. at room temperature. Measure the absorbance at 546 nm spectrophotometrically. Test substances are replaced by buffer solution for a control.[17]

**3. HPLC analysis of Quercetin**

**Plant Extraction**

10gms plant powder was extracted with 50ml Methanol at 50$^{o}$C for 4 hours. The Methanolic extracts were filtered through Whatmann No. 1 filter paper and filtrate was evaporated to dryness. Methanolic extract (10mg/ml) was used for HPLC analysis.

| | | |
|---|---|---|
| **Quercetin Standard** | **:** | 100ug/ml prepared in Methanol |
| **HPLC Condition** | **:** | |
| **Instrument** | **:** | Shimadzhu LC- Prominence 20AT |
| **Column** | **:** | C18 column 250 mm x 4.6 mm, 5u particle |
| **Mobile Phase** | **:** | Linear |
| **A** | **:** | HPLC grade Acetonitrile (60%) |
| **B** | **:** | HPLC grade Water (40%) |
| **Flow Rate** | **:** | 1.0 ml/min |
| **Injection volume** | **:** | 10ul |

**Quantification of Quercetin in plant extracts**

| | | |
|---|---|---|
| **Concentration of Standard injected** | **:** | 100µg/ml |
| **Sample concentration** | **:** | 10mg/ml |

**Formula used for quantification of quercetin in plant extract**

**Quercetin (Microgram/gram) =** Sample area / Standard area X Standard concentration injected X Dilution factor.

**4. Cytotoxicity studies using A549 cell line by MTT assay**

A549 cell line was obtained from American Type Culture Collection (ATCC) (Rockville, MD USA) (ATCC Number-CCL-185). The steps and procedure for cell culture, Thawing, Revival and Propagation of Cells were followed as described by Kangas, L. *et al*.[18]

**Procedure**

The collected cells should be about 70-80% confluency. Check the viability of the cells and centrifuge it. Take about 50,000 cells / well and seed it in 96 well plates and incubate for 24 hrs at $37^0$C, 5% $CO_2$ incubator. Add plant samples which is to be tested from 0 – 320 µg/ml (2 fold variation) concentration in RPMI without FBS & are incubated for 24 hr. Add 100µl/well of the MTT (5 mg/10ml of MTT in 1X PBS) to incubated plant samples to the respective wells and incubated for 3to 4 hours. Discard the MTT reagent by pipetting without disturbing cells and add 100 µl of DMSO to rapidly solubilize the formazan. Measure the absorbance at 590 nm.

**Calculating Inhibition**

% Inhibition = 100 – (OD of sample/OD of Control) X 100.

**5. Flow Cytometry – Cell Cycle Analysis**

**Principle**

One of the earliest applications of flowcytometry was the measurement of DNA content in cells. This analysis is based on the ability to stain the cellular DNA in a stoichiometric manner. A variety of dyes are available to serve this function, all of which have high binding affinities for DNA. The location to which these dyes bind on the DNA molecule varies with the type of dye used. The most common DNA binding dye in use today is the blue-excited dye Propidium Iodide (PI). PI is an intercalating dye which binds to DNA and double stranded RNA (and is thus almost always used in conjunction with RNAse to remove RNA). When diploid cells which have been stained with a dye that stoichiometrically binds to DNA are analyzed by flow cytometry, a “narrow” distribution of fluorescent intensities is obtained.[14]

**Reagents**

**Propidium Iodide:** Cat # P4864, Sigma; Stock 1 mg/ml, working solution are 0.05 mg/ml.

**RNase A:** Cat # 109169, Bochringer Mannhein GmbH; Stock 5 mg/ml, working solution is 0.05 mg/ml.

**Procedure**

Culture 1 x$10^6$ cells in a 6-well plate containing 2 ml of complete DMEM media. After 24h of incubation, cells are treated with or without Plant extract; 20µM Colchicine as positive control, and 1% DMSO as control in 1 ml / well of serum free DMEM media and incubate for 24 h. After 24 hr of treatment, cells were collected and, pelleted cells at 1500 rpm for 5minutes at room temperature and discard the supernatant. Resuspend the cells pellet gently in 1XPBS. Discard supernatant, resuspend pellet in 200µl of 1X PBS and fix overnight at in a 2 ml of fixing solution (70% ethanol). After 1 hr or overnight fixing, Centrifuge at 4000 rpm for 10 min at 4 °C and discard the supernatant. Cells pellet was washed two times with 2 ml of cold 1XPBS. Later, cells were incubated for 15 min at room temperature in 500µl of Propidium Iodide (PI) solution containing 0.05 mg/ml PI and 0.05 mg/ml RNase A in PBS. The percentage of cells in various stages of cell cycle in compounds treated and un-treated populations were examined using FACS Caliber (BD Biosciences,San Jose, CA).

## RESULTS AND DISCUSSION

### 1. Phytochemical analysis of *Zingiberofficinale* root extracts

**Table – 1: Phytochemical Analysis of *Zingiberofficinale* root extracts.**

| S.No | Tests | Observation | Inference |
|---|---|---|---|
| 1 | Froth formation test | Formation of stable froths was observed. | Presence of Saponins was confirmed. |
| 2 | Mayer's and Wagner's test | A brown color Precipitates was observed. | Presence of Alkaloid was confirmed. |
| 3 | Ferric Chloride test | Dark green color was developed. | Presence of Tannin was confirmed. |
| 4 | Liebermann-Burchard test | Bluish green color was observed. | Presence of Steroid was confirmed. |
| 5 | Sodium hydroxide test | Change from yellow color to colorless was observed. | Presence of Flavonoid was confirmed. |
| 6 | Ferric chloride test | Violet color was observed. | Presence of Phenol was confirmed. |
| 7 | Salkowski test | Reddish brown coloration was observed. | Presence of Terpenoid was confirmed. |

From the Table – 1, *Zingiberofficinale* root extracts qualitative analysis the phytochemical compounds such as Steroid, Terpenoids, Saponins, Alkaloids, Phenols, Tannins and Flavonoids were present.

## 2. Nitric Oxide Radical Scavenging Assay

**Table – 2: Nitric Oxide Radical Scavenging Assay.**

| Plants Name | Concentration (µg/ml) | Absorbance 546nm | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| **Control** | 0.0 | 0.5403 | 0.00 | |
| ***Standard (Curcumin)*** | 6 | 0.4992 | 7.61 | |
| | 12 | 0.4172 | 22.78 | |
| | 25 | 0.3128 | 42.11 | 25.52 |
| | 50 | 0.1680 | 68.91 | |
| | 100 | 0.1190 | 77.98 | |
| | 200 | 0.0380 | 92.97 | |
| ***Zingiberofficinale*** | | | | |
| | 5 | 0.5152 | 4.65 | |
| | 10 | 0.4684 | 13.31 | |
| | 20 | 0.3752 | 30.56 | |
| | 40 | 0.3115 | 42.35 | 32.54 |
| | 80 | 0.2175 | 59.74 | |
| | 160 | 0.1562 | 71.09 | |

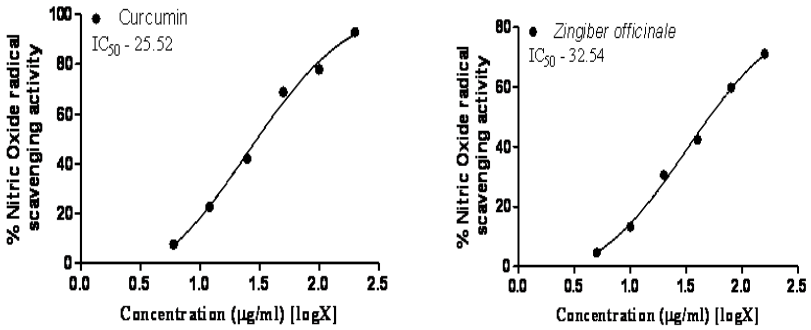


**Figure – 2: Nitric Oxide Radical Scavenging Assay.**

From Table 2 and Figure 2, the studies on nitric oxide radical scavenging assay using curcumin as standard and comparing the results with $IC_{50}$ (half maximal inhibitory concentration) values shows that the $IC_{50}$ values of *Zingiberofficinale* root extract (32.54µg/ml) were more than curcumin (25.52ug/ml). It may be due to the presence of phytochemicals which possess radical scavenging property.

## 3. HPLC analysis of Quercetin and *Zingiberofficinale* root extract

**Table – 3: HPLC analysis of Standard Quercetin.**

| S. No. | Retention. Time [min] | Area [mV.s] | Height [mV] | Area [%] | Height [%] | W05 [min] |
|---|---|---|---|---|---|---|
| 1 | 1.933 | 373.177 | 22.881 | 20.5 | 14.0 | 0.20 |
| 2 | 3.107 | 92.433 | 3.352 | 5.1 | 2.0 | 0.49 |
| 3 | 3.487 | 1296.195 | 133.916 | 71.3 | 81.6 | 0.14 |
| 4 | 4.207 | 55.054 | 3.869 | 3.0 | 2.4 | 0.22 |
| | Total | 1816.859 | 164.018 | 100.0 | 100.0 | |

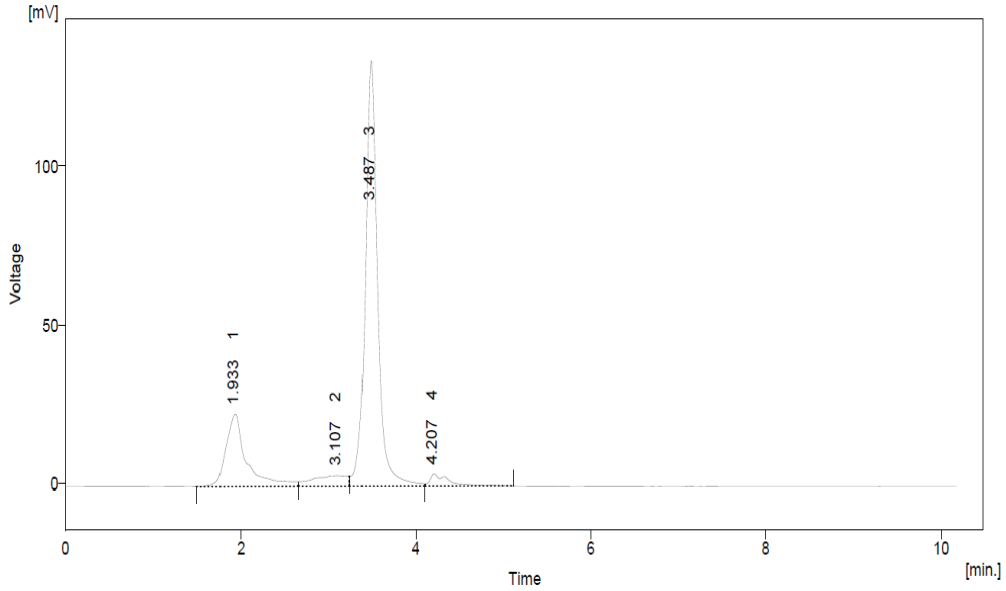


**Figure – 3: HPLC analysis of standard Quercetin.**

From Table – 3 and Figure – 3, the flavonoids were quantified at 254 nm using peak area by comparison with a calibration curve derived from the quercetin.

**Table – 4: HPLC analysis of Quercetin content in *Zingiberofficinale.***

| S. No. | Retention. Time [min] | Area [mV.s] | Height [mV] | Area [%] | Height [%] | W05 [min] |
|---|---|---|---|---|---|---|
| 1 | 1.823 | 112.102 | 10.426 | 20.8 | 25.3 | 0.14 |
| 2 | 2.257 | 230.005 | 18.953 | 42.6 | 46.0 | 0.10 |
| 3 | 3.180 | 100.141 | 6.012 | 18.5 | 14.6 | 0.12 |
| 4 | 3.340 | 97.852 | 5.782 | 18.1 | 14.0 | 0.22 |
| | Total | 540.160 | 41.172 | 100.0 | 100.0 | |

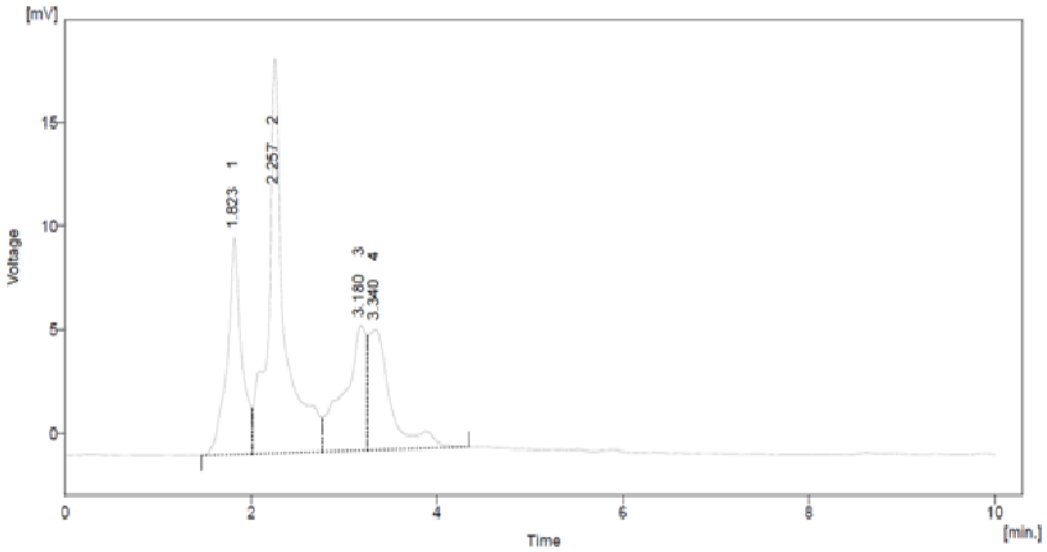


**Figure – 4: HPLC analysis of Quercetin content in *Zingiberofficinale.***

**Table – 5: HPLC analysis of Quercetin content in plant extract.**

| S.No | Sample name | Area | Quercetin (µg/g) | Quercetin (mg/g) |
|---|---|---|---|---|
| 1 | Quercetin | 1296.19 | | |
| 2 | Ginger | 97.85 | 754.91 | 0.75 |

From Table – 4 and Figure – 4, the HPLC chromatograms from root of *Zingiberofficinale* the main difference was in peak eluted at 3.4min. External flavonoids were already analyzed using HPLC method in various plant extracts. The peaks in this study shows marked increased in peak area in case of *Zingiberofficinale* root when compared with standard quercetin.

From Table – 5, the amount of Quercetin, in the sample injected was calculated. *Zingiberofficinale* root contain 0.75mg/g of quercetin. Other peaks (#1) in both the HPLC chromatogram *Zingiberofficinale* root extracts indicated the presence of other chemical constituents The present method was applicable for determining quercetin in any aerial part of plant material using HPLC technique.

## 4. Cytotoxicity studies using A549cell line by MTT assay

**Table – 6: Cytotoxic study of *Zingiberofficinale.***

| Plants name | Conc. µg/ml | OD at 590 nm | % Inhibition | $IC_{50}$ |
|---|---|---|---|---|
| | **Control** | 0.6538 | 0.00 | |
| ***Zingiberofficinale*** | 10 | 0.6194 | 5.26 | 79.57 |
| | 20 | 0.5541 | 15.25 | |
| | 40 | 0.4772 | 27.01 | |
| | 80 | 0.3398 | 48.03 | |
| | 160 | 0.2438 | 62.71 | |
| | 320 | 0.1446 | 77.88 | |

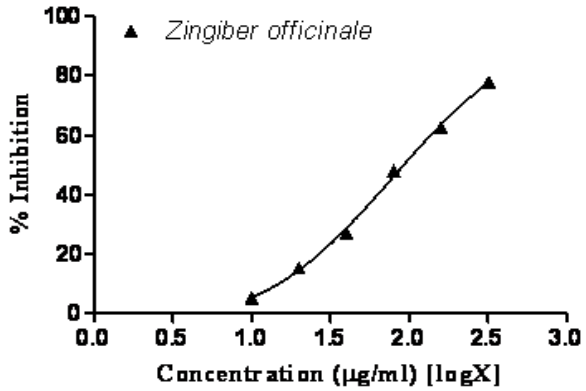


**Figure – 5: Cytotoxic study of *Zingiberofficinale.***

From Table 6 and Figure 5, the studies on A549 cancer cell line using MTT assay after completing sub cell culture collected the cells when they reach about 70-80% confluency showed $IC_{50}$ value 79.57µg/ml. This suggests that *Zingiberofficinale* root extract has significant dose dependent inhibition growth of A549 cells. Hence the *Zingiberofficinale* root extract was found to be a powerful anti cancerous component.

**5. Cell Cycle of A549 cell line using flow cytometry**

**a. Control 1%DMSO**

**Table – 7: Flow Cytometry plots of cell treated with 1% DMSO as Control.**

| Marker | Left | Right | Events | %Gated | %Total | Mean | CV |
|---|---|---|---|---|---|---|---|
| All | 0 | 1023 | 10000 | 100.00 | 80.03 | 221.60 | 28.23 |
| $SubG^0$ | 68 | 163 | 16 | 0.16 | 0.13 | 159.56 | 1.65 |
| $G^0/G^1$ | 163 | 225 | 8055 | 80.55 | 64.47 | 193.58 | 4.24 |
| S | 228 | 344 | 765 | 7.65 | 6.12 | 283.85 | 12.40 |
| $G^2M$ | 344 | 421 | 1116 | 11.16 | 8.93 | 375.40 | 4.11 |

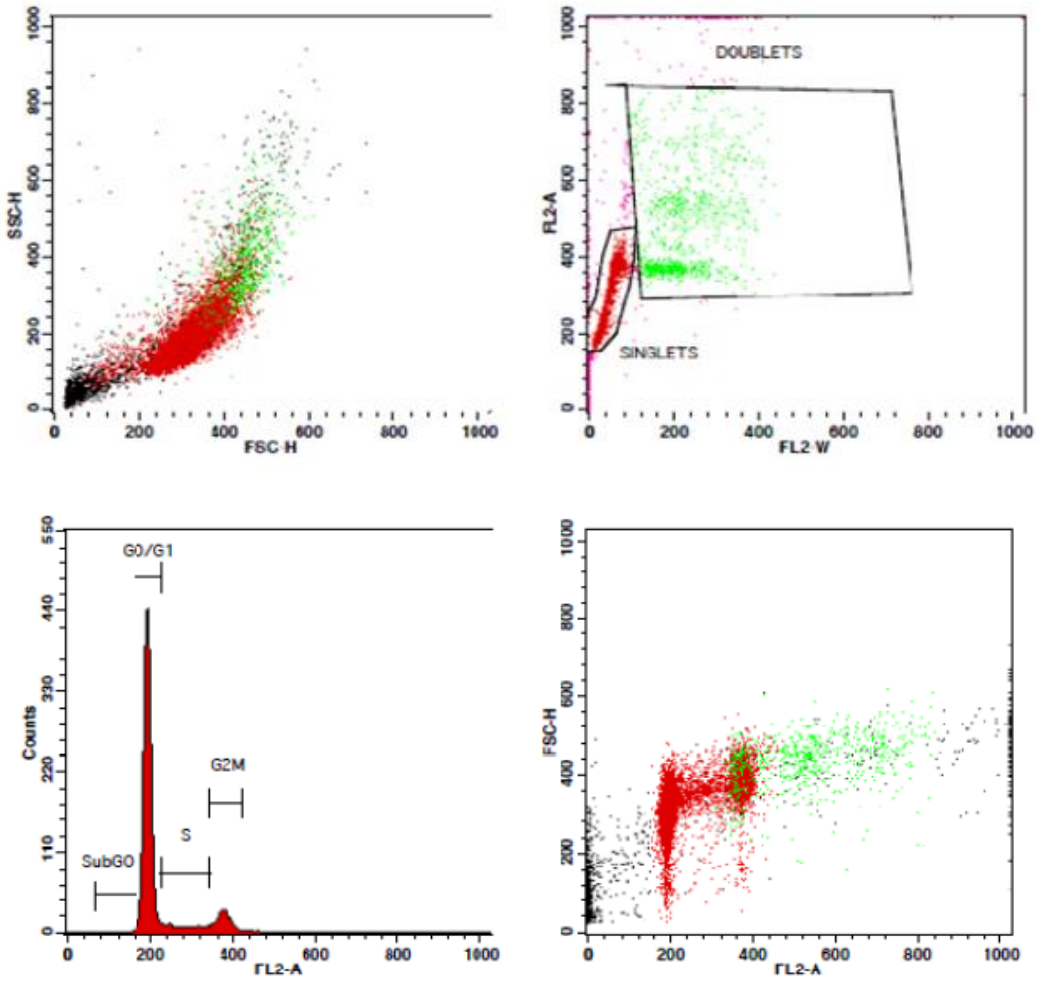


**Figure – 6: Control 1%DMSO.**

**b. Effect of Colchicine (20µM) on Cell line**

**Table – 8: Flow Cytometry plots of cell treated with20µM of Colchicine.**

| Marker | Left | Right | Events | %Gated | %Total | Mean | CV |
|---|---|---|---|---|---|---|---|
| All | 0 | 1023 | 9810 | 100.00 | 63.86 | 315.23 | 27.57 |
| $SubG^0$ | 90 | 176 | 0 | 0.00 | 0.00 | --- | --- |
| $G^0/G^1$ | 174 | 242 | 3475 | 35.42 | 22.02 | 207.58 | 5.47 |
| S | 241 | 344 | 1222 | 12.46 | 7.96 | 298.23 | 10.29 |
| $G^2M$ | 344 | 456 | 5153 | 52.53 | 33.55 | 391.70 | 4.87 |

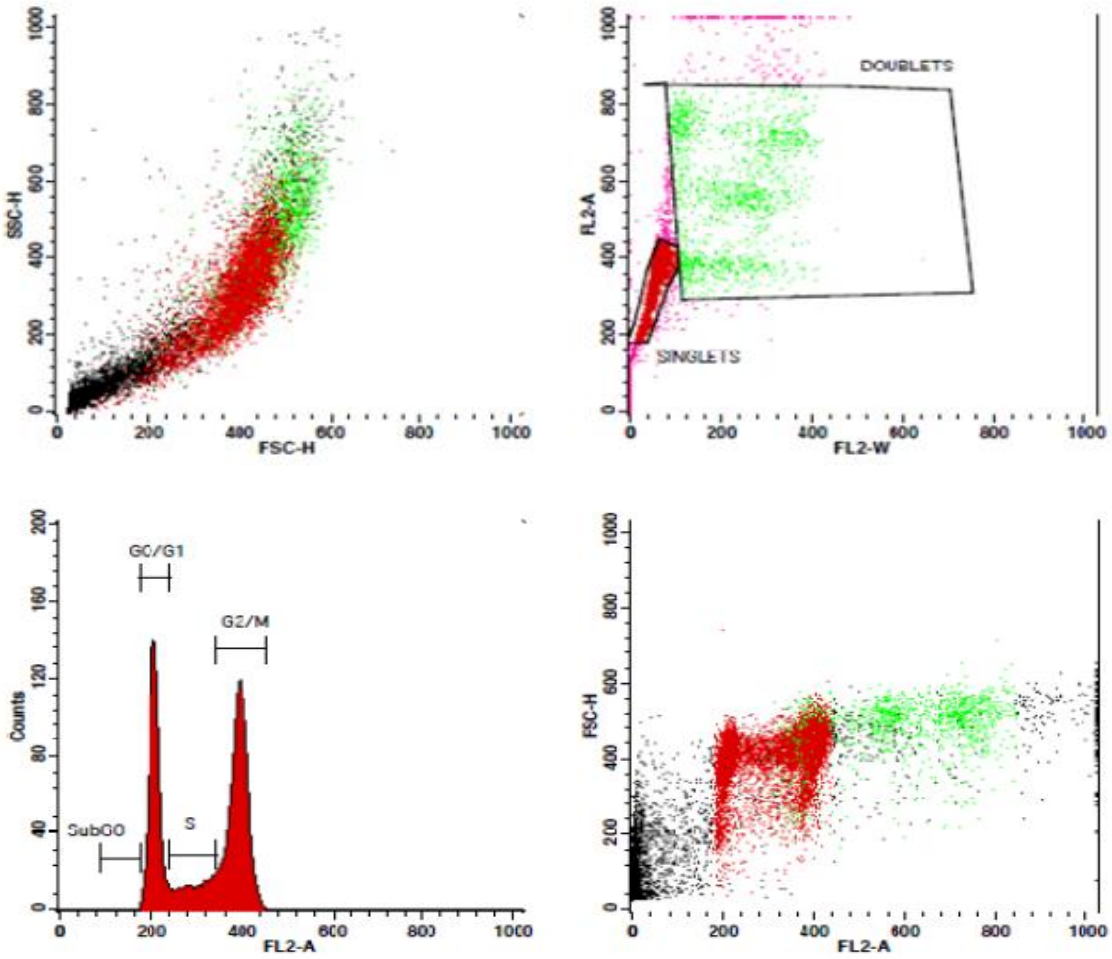


**Figure – 7: Effect of Colchicine (20µM) on Cell line.**

**c. Effect of Plant Extract on Cell line**

**Table – 9: Flow Cytometry plots of cell treated with Plant Extract.**

| Marker | Left | Right | Events | %Gated | %Total | Mean | CV |
|---|---|---|---|---|---|---|---|
| All | 0 | 1023 | 10000 | 100.00 | 25.68 | 267.46 | 33.33 |
| $SubG^0$ | 23 | 151 | 1438 | 14.38 | 3.69 | 133.65 | 7.44 |
| $G^0/G^1$ | 151 | 236 | 2640 | 26.40 | 6.78 | 189.92 | 12.90 |
| S | 236 | 290 | 1102 | 11.02 | 2.83 | 261.56 | 6.21 |
| $G^2M$ | 290 | 448 | 4910 | 49.10 | 12.61 | 348.60 | 8.24 |

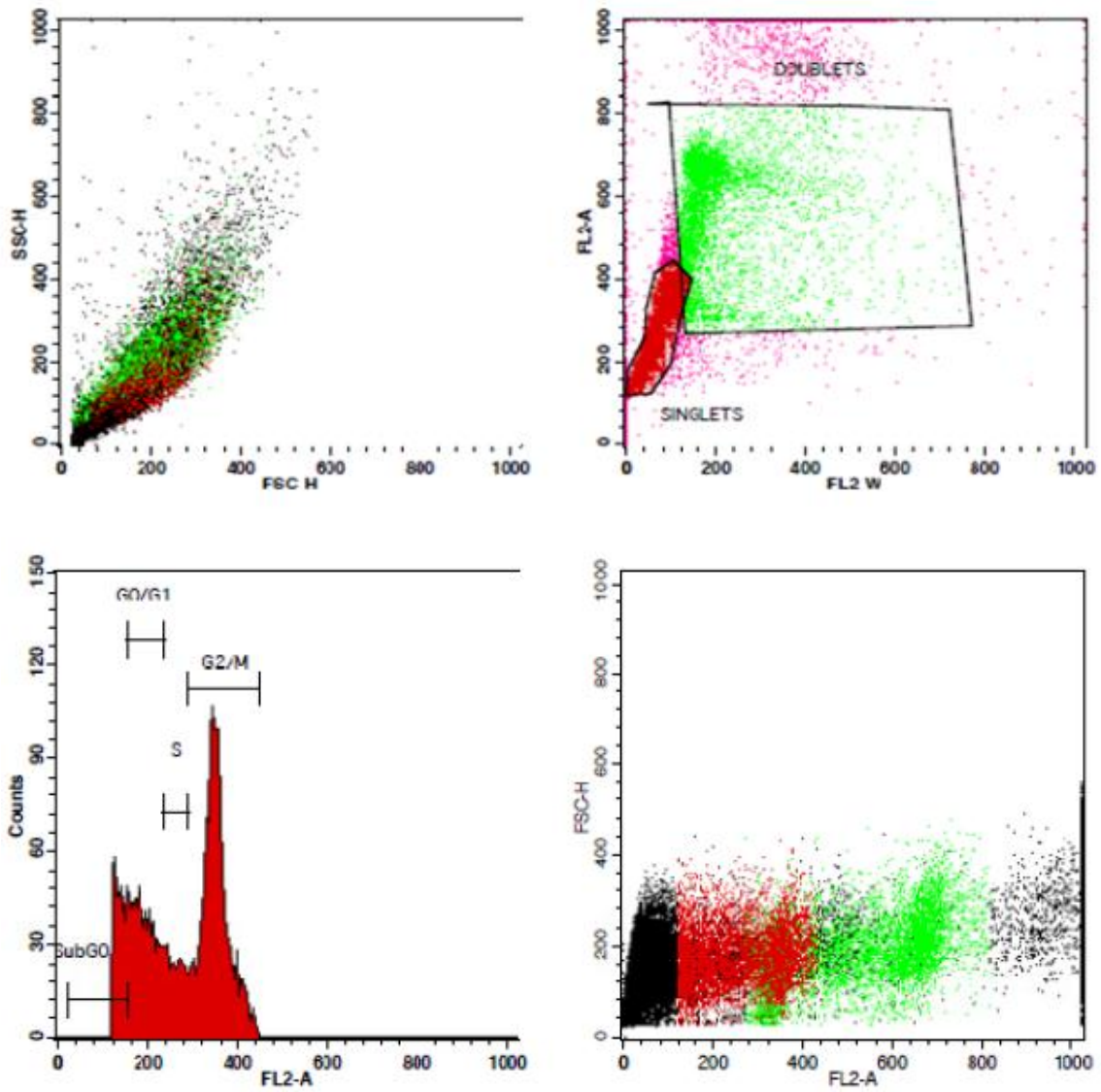


**Figure – 8: Effect of Plant Extract on Cell line.**

From the Table – 7, 8, 9 and Figure – 6, 7, 8 the plant extract treatment has arrested 49.10% at G2M phase of cell cycle, compare to untreated control cells (11.16%). Colchicine has showed 52.53 % of cell cycle arrest at G2M phase. This result suggests plant extract showing very effective cell cycle arrest at G2M phase and at the same time it does induces the apoptosis.

## CONCLUSION

Our results in accordance with the above findings shows that *Zingiberofficinale* root possess maximum phytochemical component that lend credibility for being good radical scavengers. The maximum Nitric oxide scavenging $IC_{50}$ valve of standard Quercetin and

*Zingiberofficinale* was found to be 25.52 and 32.54 respectively. The MTT assay found that there were cytotoxic effects with increasing concentration onA549 cell line from 10µg to 320µg concentration when compared to the untreated A549 cells. Flowcytometry analysis showed the plant extracts arrest 49.10% at G2M phase of cell cycle which clearly indicates the effectiveness of inducing apoptosis and hence this plant extracts can be taken up for further studies.

**ACKNOWLEDGEMENT**

The authors are thankful to Skanda Life Sciences Private Limited, Bangalore for providing necessary facilities to complete this project successfully.

**REFERENCES**

1. Mohammad Mijanur and Md. Asaduzzaman. Anticancer potential of south asian plants, Nat prob bio prospact, 2013; 3(3): 74-78.
2. American Joint Committee on Cancer (AJCC). AJCC staging manual (6th ed.). 2002; New York; Springer-Verlag.
3. Giard DJ, Aaronson S A, Todaro G J, Arnstein P, Kersey J H, Dosik H, Parks W P. Invitro cultivation of human tumors: Establishment of cell lines derived from a series of solid tumors. *J. Natl. Cancer Ins*, 1973; 51: 1417-1423.
4. Foster K A. Oster C G, Mayer M M, Avery M L, Audus K L. Charecterization of the A549 cell line as a Type II Pulmonary Epithelial Cell Model for Drug Metabolism. Exp. Cell Res, 1998; 243: 359-366.
5. Grzanna R, Lindmark L, Frondoza C. Ginger - A herbal medicinal product with broad anti-inflammatory actions. Journal of Medicinal Food, 2005; 8(2): 125-132.
6. Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M *et al.* Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods.\Food Chemistry, 2005; 91(4): 621-632.
7. Kawai T. Anti-emetic principles of Magnolia obovata Bark and *Zingiberofficinale* Rhizome, Planta Medica, 1994; 60(1): 17-20.
8. Govindarajan VS. Ginger: Chemistry, technology, and quality evaluation: Part 1 & 2. Crit Rev Food Science & Nutrition, 1982a & b; 17: 1-96 & 189-258.
9. Ali BH, Blunde G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiberofficinale Roscoe*): A review of recent research. Food and Chemical Toxicology, 2008; 46: 409-420.

10. Kim, S.O., Kundu, J.K., Shin, Y.K., Park, J.H., Cho, M.H., Kim, T.Y., Surh, Y.J. [6]-Gingerol inhibits COX-2 expression by blocking the activation of p38 MAP kinase and NF-kappaB in phorbolesterstimulatedmouse skin. Oncogene, 2005; 24: 2558–2567.
11. Rhode, J., Fogoros, S., Zick, S., Wahl, H., Griffith, K.A., Huang, J. & Liu, J.R. Ginger inhibits cell growth and modulates angiogenic factors in ovarian cancer cells. *BMC ComplAltern*, 2007; 20: 11-76.
12. Manju,V. &Nalini, N. Chemopreventive efficacy of ginger, a naturally occurring anticarcinogen duringthe initiation, post-initiation stages of 1,2 dimethylhydrazine-induced colon cancer. *ClinChim Acta*, 2005; 358: 60–67.
13. Ranjani Ramakrishnan. Anticancer properties of ZingiberOfficinale- Ginger; A Review. International Journal of Medicine andPharmaceutical Sciences, 2013; 3(5): 11-20.
14. Alan J. Potter, Katherine A. Gollahon, Ben J.A. Palanca, Mary J. Harbert, Young M. Choi, Alexander H. Moskovitz, John D. Potter, Peter S. Rabinovitch. Flow cytometric analysis of the cell cycle phase specificity of DNA damage induced by radiation, hydrogen peroxide and doxorubicin. Carcinogenesis, 2002; 23(3): 389–401.
15. Hemant RJ, Bhutani KK. Antioxidant properties of Indian Medicinal plants. Phytother. Res, 2002; 16(8): 771-3.
16. Segelman AB, Fransworth NR, Quimbi MD, L Loide. Biological and phytochemical evaluation of plants. Pub med, 1969; 1: 52-58.
17. Shreejayam, Rao M N A. Nitric oxide scavenging by curcuminoids. *J. Pharm. Pharmacol*, 49; 105-107.
18. Kangas, L. *et al*. Bioluminescence of cellular ATP: A new method for evaluating cytotoxic agents in vitro. *Med. Biol*, 1984; 62: 338–43.