CHAPTER-5

DISCUSSION
5. DISCUSSION

The usage of herbal medicine has its origin since human civilization based on their experience the herbal practitioners of local area were started using formulations prepared from medicinal plants for the treatment of human disease. The preparations like powders, tinctures, poultices, and decoctions and also the isolated drugs were used to prescribe for the patients. Later they have identified the drugs such as anticancer drugs vinblastine and taxol, cardiotonics like digoxin, antitussives like codeine analgesics like morphine, reserpine a antihypertensives drug, quinine and artemisinin are the antimalarials drugs derived from plant origin (Ramawat et al., 2009: Kumar et al., 2015).

5.1 EXTRACTION

Extraction is the process of separation of bioactive compounds from different parts of the plant such as leaf, root, bark and stem bark etc. using non reactive solvents like petroleum ether, chloroform, hexane, ethanol, methanol and water etc. by referring standard protocol. The extract obtained through above process contains active ingredients like alkaloids, glycosides, terpenoids, flavonoids etc in the form of impure liquid, semi-solid liquid and dry powder form that can be evaluated to know pharmacological properties of the compounds (Handa et al., 2008). In present study the stem bark powder of Ficus krishnae was subjected to the hot soxhlet extraction by using different solvent such as petroleum ether, chloroform, methanol and aqueous. The obtained extracts were collected, dried and stored it for further use.

5.2 QUALITATIVE SCREENING OF PHYTOCONSTITUENTS

The pharmacological activity of plant extract is due to presence of secondary metabolites in plant. Therefore, it is important to known the therapeutic properties of
plant based compounds by screening of plant and plant parts such as leaf, stem bark, fruits, bark etc both qualitatively and quantitatively. The stem bark extract of *Ficus krishnae* was successfully extracted by soxhlet extraction with four different solvents such as petroleum ether, chloroform, methanol and aqueous and screened for qualitative analysis primary and secondary metabolites of *F. krishnae* stem bark extracts the results revealed the presence of protein, carbohydrates, glycosides, saponins, sterols, terpenoids, flavonoids and alkaloids. Petroleum ether and chloroform extracts have responded positively to steroids, terpenoids, protein carbohydrates, alkaloids whereas glycosides are present only in chloroform extract. The methanol extract has shown positive response for the test of protein, carbohydrates, glycosides, saponins, sterols, terpenoids, flavonoids, tannins, phenols, glycosides and alkaloids. The aqueous extract exhibits positive for the presence of flavonoids, tannins, phenols, alkaloids, protein and carbohydrates exhits in the stem bark we take previlage to first report on presence of above components in *Ficus krishnae*. The present investigation results very much correlates with the previous studies conducted on another species of *Ficus religiosa* where the presence of saponins, phenols, alkaloids, protein, flavanoids, terpenoids and tannins in the leaves (Prakash et al., 2017). Sudhakar et al., (2012) also carried out the phytochemical screening of *Ficus glorimeta* ethanol leaf extract & the results revealed the presence of steroids, phenols, tannins, quenones and coumarins. These above literature findings are in accordance with our results. Further the presence of different primary and secondary metabolites in plant extract is directly related to potential pharmacological activity of the *Ficus krishnae*.
5.3 ANTIMICROBIAL ACTIVITY

Today the whole world is accepted the importance of medicinal plants to be used as alternative medicines for treating various microbial infections because of increase in development of resistivity against antibiotics (Subhash et al., 2016). The antimicrobial activity of *Ficus benghalensis var. krishnae* prop root extract was studied previously by Subhash et al., (2016) against *E.coli* and *Pseudomonas aurignosa* indicated that the methanol extract formed 15mm zone of inhibition.

In the present study the Petroleum ether (PE) extract has shown zone of inhibition about 15 mm for *Escherichia coli* (MTCC 45) followed by 14 mm zone of inhibition on *Shigella dysenteriae* (clinical isolate), *Salmonella typhimurium* (MTCC 98) and about 13 mm zone of inhibition against *Enterococcus faecalis* (ATCC 29212) and 12 mm of zone of inhibition against *Enterobacter aerogenes* (MTCC 111) and also for *Staphylococcus aureus* (ATCC 29122). But comparatively less about 11 mm zone of inhibition was observed in *Aspergillus niger* (MTCC 282).

Chloroform extract of *Ficus krishnae* inhibition the growth of *Staphylococcus aureus* (ATCC 29122) and *Aspergillus niger* (MTCC 282) by forming 14 mm zone was observed. Whereas has for *Enterobacter aerogenes* (MTCC 111), *Enterococcus faecalis* (ATCC 29212) and *Shigella dysenteriae* (clinical isolate) it was about 13 mm of zone of inhibition has observed but 12 mm zone of inhibition was measured in *Salmonella typhimurium* (MTCC 98) and *Escherichia coli* (MTCC 45).

The methanolic extract has shown significantly zone of inhibition in both *Aspergillus niger* (MTCC 282) and *Salmonella typhimurium* (MTCC 98) by forming 17 and 16 mm of zone of inhibition respectively. *Enterobacter aerogenes* (MTCC 111), *Escherichia coli* (MTCC 45), *Shigella dysenteriae* (clinical isolate) it was 15
mm of zone of inhibition has recorded. Whereas Staphylococcus aureus (ATCC 29122) it was 13 mm and for Enterococcus faecalis (ATCC 29212) it was about 12 mm of zone of inhibition.

In case of aqueous extract of Ficus krishnae the zone of inhibition was measured about 15mm for Escherichia coli (MTCC 45) and 14 mm of zone of inhibition for Enterococcus faecalis (ATCC 29212). The inhibition of about 13 mm for Salmonella typhimurium (MTCC 98) and 10 mm for Aspergillus niger (MTCC 282). 9 mm for Staphylococcus aureus (ATCC 29122), 8 mm for Shigella dysenteriae (clinical isolate) and 6 mm for Enterobacter aerogenes (MTCC 111) was observed. Similarly the results of the study conducted the highest zone of inhibition was 15 mm for Aeromonas hydrophila, 14 mm for Staphylococcus aureus, Enterobacter aerogenes is about 16 mm, 13 mm for Pseudomonas aeruginosa and Streptococcus pyogenes were correlates with the results obtained in our research (Rajiv and Sivraj 2012)

Ramakrishnaiah and Hariprasad (2013) research on methanolic extract of Ficus religiosa has exhibited significant zone of inhibition for S.aureus it was about 28 mm and in E.coli it was 16 mm and for Pseudomonas aeruginosa 12 mm zone of inhibition. The ethyl acetate latex extract of Ficus carica has expressed strong zone of inhibition of about 13 mm for Proteus mirabilis, 11-12 mm for Pseudomonas aeruginosa, 11 mm for Echerchia coli and 10-12 mm zone of inhibition for Enterococcus Fecalis was observed (Aref et al., 2010).

5.4 ANTIOXIDANT ACTIVITY

Continuous biological and chemical reaction occurs in the body of an organism these reactions requires reactive oxygen species that contains hydroxyl and
superoxide ions free radicals. These free radicals are the compounds generated in the body by regular metabolic activities. The free radicals will induces stress that damage DNA, protein and lipids in cells and tissue leading to various disease like cancer, cardiovascular, inflammation and aging (Halliwell 1995; Donald and Cristobal 1987).

In the present study the antioxidant activity of Ficus krishnae stem bark extracts was determined by DPPH scavenging activity, ABTS scavenging activity, reducing power assay and phosphomolybdenum assay.

The DPPH radical scavenging activity of petroleum ether and chloroform extracts of F. krishnae stem bark was ranged from 16.87% to 60.50% and 22.98% to 61.04% respectively, whereas scavenging activity of methanol extract found to be 46.97% to 97.74% at 5-100 μg/mL and in aqueous extract it was 39.07-72.01% of scavenging activity at 5-100 μg/mL of aqueous extract respectively, ascorbic acid has showed 86.35% to 98.20% inhibition. The IC50 values of PE, CH, MH, AQ and ascorbic acid 78 μg/mL, 82 μg/mL, 7 μg/mL, 24 μg/mL and 2 μg/mL respectively.

The ABTS scavenging activity of petroleum ether, chloroform, methanol and aqueous extract have shown successively like PE>CH>MH>AQ exhibiting good scavenging activities with their IC50 values was found to be 4 μg/mL, 4 μg/mL, 4 μg/mL, 5 μg/mL respectively. Where as ascorbic acid has shown 4 μg/mL. Higher scavenging of DPPH and ABTS free radical scavenging activity was noticed with methanolic extract followed by aqueous, chloroform and petroleum ether extracts. The higher concentration of phenols and flavonoids in methanol extract which are responsible for high antioxidant capacity. There is a significant relation between antioxidants activity of phenolic content as described by Awika et al., (2003) where the positive correlation of phenol was observed by oxygen radical absorbance.
capacity (ORAC), DPPH and ABTS assays. According to Meir et al., (1995) reported that phenolic compounds are mainly responsible for the reaction between antioxidant molecules and radicals finally they scavenge radical through hydrogen ion donation by decolourization from purple to yellow. Our results are also correlated with the Londonkar and Shivasharanappa (2014) study on *Ficus glorimeta* methanol extract where the IC$_{50}$ value of 7.23 and 9.89 mg/mL of DPPH and ABTS assay respectively.

Phosphomolybdenum assay based on the reduction of MO (VI)-MO (V) by the extract and formation of a MO (V) complex at acidic pH and the formation of a green phosphate/Mo (V) complex with a maximal absorption at 695 nm. In the present study shown the highest reducing potential was observed at a concentration 100 µg/mL exhibited by MH followed by CH, AQ and PE where the absorbance values was recorded as 0.397±0.05, 0.078±0.02, 0.068±0.04 and 0.048±0.02 respectively, but the BHT has shows the absorbance of 0.605±0.05 at the same concentration. It is confirmed that the methanol extract of *Ficus krishnae* exhibited potential antioxidant activity.

The reducing power of the extracts is mainly due to their redox property, which play key role in absorbing and stabilizing free radicals, quenching singlet and triplet oxygens. According to Oyaizu (1986) the reductive capacity of the compounds can be measured by transfer of electron from Fe3+ to Fe2+ that occurs in the different extracts. There is direct correlation between antioxidant activities and reducing power of various isolated compounds from plant, where reduction occurs in the presence of reductones, which break the free radical chain by donating hydrogen and also by inhibiting the formation of peroxide (Tanaka et al., 1999).
The results obtained in our study will also positively correlate with the previous studies conducted by Yadav et al., (2011) in-vitro antioxidant activity of methyl alcohol extract of *Ficus benghalensis* through DPPH, phosphomolybdenum, ferric chloride has showed the IC$_{50}$ values 28.63± 0.16 µg/mL, 31.86±0.12 µg/mL and 49.82±1.00 µg/mL respectively.

5.5 ANTI-INFLAMMATORY ACTIVITY

Inflammation is natural defense mechanism of a body in which the protein loses its primary and secondary structure leading to the formation of inflammation by various external factors like stress, organic solvents, heat, acids reaction etc., it is associated with causing vascular permeability, membrane alteration and pain (Leelaprakash and Dass, 2010). In the present study chloroform extract were shown the significant inhibition with IC$_{50}$ value 120µg/mL, for methanol extract (MH) 550µg/mL, for petroleum ether (PE) 650µg/mL and aqueous it was about 700µg/mL compared to the standard Diclofenac sodium which has shows the IC$_{50}$ values 19µg/mL.

Our positive findings are correlated with Mahesh et al., (2013) from the in-vitro anti-inflammatory effect and results shown that the methanol extract of bark has shown about 67.24±1.01 % inhibition activity compared to standard.

5.6 EXTRACTION, ISOLATION AND PURIFICATION OF COMPOUND

In the present study *Ficus krishnae* stem bark powder was subjected for extraction with various solvents such as petroleum ether, chloroform, methanol and aqueous. Among all solvent extracts the methanol extract contains rich source of active components. Hence, the methanol extract was used for isolation and separation of pure compound from *Ficus krishnae* through chromatographic studies.
Column chromatography is the simple and basic technique commonly used in the separation and purification of bioactive compounds from only plant extracts. Thin layer chromatographic (TLC) technique used to separate and identify active compounds of interest and has advantage for low cost, required short time and multiple detections in a same plate. In the present study the methanol extract tested for thin layer chromatography by using chloroform: methanol (9:1) solvent system, developed spot Rf value 0.8. These are further separated and purified by using column chromatography over silica gel; fractions were collected and checked for its purity by thin layer chromatography (TLC) using chloroform: methanol (9:1). The similar Rf value fractions are pooled and evaporated for conducting further tests like physicochemical and spectroscopic studies for the isolation of pure compounds.

Similarly Asha and Kumar (2015) were also performed the thin layer chromatography study to identify the bioactive compound from Acorus calamus Linn extract.

The physicochemical observation indicates that the isolated compound is a yellow colour liquid with a boiling point of 204°C, soluble in methanol and DMSO. Further the structural characterization was done by NMR, Mass spectrometry and FTIR technique.

Nuclear magnetic resonance (NMR) is one of the important techniques followed by researchers to identify the unknown compounds. The basic principle of NMR is mainly dependent on the magnetic properties of atomic nuclei like carbon atoms, hydrogen atoms, protons and also carbons isotope (Kemp 1991).

$^{13}$C-NMR spectrum data analysis reveals that the compound isolated from methanolic extract of Ficus krishnae consists of methane ($\delta c$ 123.2), one oxygenated
methylene (§c 59.2), five methyls (§c 22.64, 22.73, 19.76, 19.72 and 1.66), one olefinic quaternary (§c 139.9), nine methylene and three methane signals. It appears that the compound contains 20 carbons.

In the $^1$H NMR spectrum resonance at 5.32 ppm indicated the presence of double bond at 4.14 ppm which indicates the presence of oxygenated methylene group (-OCH$_2$), four methyl doublet signals were identified as 0.87, 0.85, 0.85, 0.84 ppm including several methylene and methane signals, this data indicating that the compound contain 40 hydrogen atoms.

Mass spectrometry was the best technique to identify the relative molecular mass (molecular weight) of bioactive compound, this technique applied only for organic compounds because of bombardment of electrons that converts into energetic charged ions this gives rise to molecular mass of compound (Christophoridou et al., 2005). In the current study mass spectrum (GC-MS) of the compound has shown maximum peak at 296.6.

Fourier transform infrared spectroscopy (FTIR) is technique applied to know the chemical component and structure of compound, it involves absorbance of infrared by the molecules with a vibrational change due expose to infra-red radiation. The frequency of vibration creates a peaks that help in the detecting the character of molecules (Urbano et al., 2006).

In the present study FTIR spectrum of isolated compounds has shown the broad peaks at 3200-3600 cm$^{-1}$ it is due to OH stretch, the another broad peak appeared at 2960 cm$^{-1}$ due to alkyl C-H stretching. The peak around 1350 cm$^{-1}$ will correspond to C=C stretching and at 1027 cm$^{-1}$ is due to C-O group.
Similarly Bang et al., (2002) have observed the IR bonds for hydroxyl group (3332 cm\(^{-1}\)) and olefine (1669 cm\(^{-1}\)) in the IR spectrum (CHCl\(_3\)) and molecular ion peaks at m./z 296. In the \(^1\)H NMR spectrum (400 MHz, CDCl\(_3\)), an olefinic methane (§5.40, tq), an oxygenated methylene (§4.14, d) and an allyl methylene (§1.99) peaks were observed. In high magnetic field region, an allyl singlet methyl (§1.66) and four doublet methyls (§0.84, 0.85, 0.87 (×2)) including several methane. \(^{13}\)C-NMR spectrum that contains 20 signals consisting of one olefinic quaternary (§c 140.14) and methane (§c 123.11), one oxygenated methylene (§c 59.34), five methyls (§c 22.69, 22.59, 19.72, 19.68, 16.13), nine methylene and three methine signals. They obtained a molecular formula as C\(_{20}\)H\(_{40}\)O for the unknown compound and it was identified as terpene. The above IR spectral studies methods and data were supported to analyse and indentify our compound.

The mass spectrometry data of isolated compound obtained in the present research revealed the mass of 296.6 which is a nearest value to the terpene Phytol (296.53). Hence, the molecular formula for our compound can be drawn as C\(_{20}\)H\(_{40}\)O this confirms that isolated compound is Phytol.

Phytol is an acyclic alcoholic diterpene, its IUPAC system named it as 3, 7, 11, 15-tetramethylhexadec-2-en-1-OL, found as essential oils of aromatic plants. It is a product of chlorophyll metabolism synthesized in plant and used as a precursor for the production of synthetic vitamin E and vitamin K (Rotani and Volkman, 2003).

5.7 ANTIMICROBIAL ACTIVITY OF PHYTOL

The isolated compound phytol exhibits potential antibacterial activity against the tested organisms like *Shigella dysenteria* (clinical isolates) it is about 15 mm and 17 mm zone of inhibition at the dose of 25 mg/mL and 50 mg/mL respectively. The
zone of sensitivity of *Enterococcus faecalis* (ATCC 29212) was about 12 mm and 16 mm. *Escherichia coli* (MTCC 45) has shown the 14 mm and 16 mm zone of inhibition, whereas phytol tested on *Staphalococcus aureus* (ATCC 29122) has shown the moderate activity by forming 10 mm and 12 mm of zone of sensitivity at above concentration. Similarly to our results the anti-microbial activity (MIC$_{50}$) of phytol has significant growth inhibitory effect on *E.coli, C.albicans* and *A.niger* are 62.5 mg/mL and S. aureus was > 1000 mg/mL (Ghaneian et al., 2015). Eluchie et al., (2016) have also investigated the antibacterial activity of phytol, where the results indicated that the *Pseudomonas aeroginosa* and *Staphalococcus aureus* are highly susceptible to phytol at 62.5 µg/mL and 31.25 µg/mL respectively. Thus it supports that the phytol has significantly inhibiting action on growth of bacteria in the media and it can be used as potential antibacterial drug against both Gram positive and Gram negative bacteria.

### 5.8 ANTIOXIDANT ACTIVITY

#### 5.8.1 IN-VITRO ANTIOXIDANT ACTIVITY OF PHYTOL

Reactive oxygen species are strongly involved in the chemical reaction with both macromolecules such as protein, lipids, DNA and causes damage to cell membrane, DNA denaturation or mutation (Fito et al., 2007). In present study the phytol had shown the high rate of ABTS scavenging activity, it was about 99.57% of inhibition at the 100 µg/mL concentration and the IC$_{50}$ values is 13 µg/mL, followed by DPPH scavenging activity, it was recorded as percentage of inhibition 66.09% at the concentration of 100 µg/mL and its IC$_{50}$ values of is 87µg/mL. The phytol acts as antioxidant molecule by inhibiting the formation of free radicals, this action may be due to presence of hydroxyl group in the chain. Hence, it reacts with free radicals by donating the hydrogen atom with unpaired electron and converting free radicals into
less reactive species (Guimaraes et al., 2010; Lima and Cardoso 2007). The above research work can be correlated with our present study to confirm the antioxidant properties of phytol (Santos et al., 2013).

However the hepatoprotection study of phytol reveals the significant recovery in tissue architecture by histopathological evaluations after phytol treatment. In the present study the liver enzyme activities like SOD, CAT and GSH in the CCl₄ treated groups has significantly decreased compared with normal control due to phytol. Where the phytol treated animal group have shown the increases of antioxidants enzymes liver the SOD is increased from 4.48±0.24 to 10.62±0.162 U/mg, whereas CAT was increased from 25.42±0.66 to 50.64±0.28 U/mg and GSH from 26.44±0.12 to 58.66±0.86 nM/gm. The phytol has been shown to well-protestant against the acute hepatotoxicity by by inhibiting 59.89±0.73% in DPPH assay and ABTS assay has showed 62.79±1.99 at 7.2µg/mL and also there is an increases in the SOD level at 75 µg/mL by 63..4%, whereas CAT activity was increased by 136.8% and GSH at the dose of 75µg/mL has exhibited increased by 44.07% (Costa et al., 2016).

5.9 ANTI-INFLAMMATORY ACTIVITY OF PHYTOL

Inflammation results in the oxidative stress evoking formation of free molecules. The inflammation includes complex of problems like edema, pain, fever, erythema and cell migration at the site of edema. Many synthetic drugs are used for the treatment of inflammation but these drugs will cause side effects such as bleeding, formation of gastrointestinal ulcers and renal disfention (Junior et al., 2011).

In the present study in vivo anti-inflammatory activity of phytol has shown significantly reduction of inflammation from 0.36±0.02 mL in rat paw edema. Similarly the research work Silva et al., (2013) revealed that the phytol at the 75
mg/kg has high inhibitory effect expressing about 5.8% (0.026±0.002 mL) and it was dose dependent manner. Anti-inflammatory activity of different concentration of phytol showed a strong reduction of paw edema about 68% (Phatangare et al., 2017). The above literature data very much correlated with the experimental data obtained in study proving that the phytol acts as potential anti-inflammatory agent.

5.10 ANTICANCER ACTIVITY OF PHYTOL

More then 70% of the ovarian cancer is detected at the metastasis stage due to some gynecological complication. Even after the availability of modern techniques to diagnose the cancer but they fail to detect ovarian cancer at preliminary stage to stop women mortality (Yap et al., 2009).

In the present study the phytol was analyzed for its cytotoxicity by MTT assay in ovarian cancer cells (SKOV-3) treating at phytol to SKOV3 cells at different concentration. Demonstrated that it has decreased the cell viability in dose dependent manner with IC\textsubscript{50} value 177.7 µg/mL. This clearly indicates that the phytol contain potential quality to be used as a drug against ovarian cancer cells.

Sheeja et al., (2016) conducted the cell cytotoxicity effect by MTT assay on breast cancer cell lines like MCF-7 it potentially inhibited the cell growth and cell viability with IC\textsubscript{50} values 125 µg/mL of phytol. Hence, the result obtained in the present investigation on phytol is correlated to the results of above researcher. Similarly the study on phytol through MTT assay at in vitro condition against seven tumors cell lines shown high and low efficacy against breast adenocarcinoma MCF-7 cells and prostate adenocarcinoma PC-3 cells with IC\textsubscript{50} 8.79 ± 0.41mM and 77.85 ± 1.93mM respectively. Whereas the phytol effect on the other tumor cell lines like HeLa, HT-29, A549, Hs294T and MDA-MB-231 has shown IC\textsubscript{50} value ranging from
15.51 to 69.67 mM and least activity shown in foetal lung fibroblast MRC-5 and IC$_{50}$ 124.84 ± 1.59mM of was observed (Pejin et al., 2014).

Apoptosis or programmed cell death (PCD) is an important part of normal physiological development, which maintains the balance between cell proliferation and cell death. Apoptosis remove the aged and damaged cells by intrinsic pathways usually through mitochondrial and extrinsic pathway by receptor mediated. Any changes in the apoptosis lead to cause several diseases such as cancer, AIDS etc (Indran et al., 2011; Parthiban et al., 2014).

In present study the apoptosis activity of phytol on ovarian cancer cells was studied by flow cytometry methods has resulted in apoptosis at the IC$_{50}$ concentration at 160 and 320µg/mL, with high dose of phytol around 50% of apoptosis has been observed within 24 hours. It is agreed that the phytol induces apoptosis through extrinsic pathway by activating the CASPASE-3 and CASPASE-8 genes.

The study on Cassia auriculata leaf extract for cytotoxic effect in MCF-7 and Hep-2 (larynx carcinoma) has induced apoptosis in both cell lines by reducing the expression of anti-apoptotic Bcl-2 protein and up-regulation of pro-apoptotic Bax protein (Prasanna et al., 2009). Another research work was conducted by Zhou et al., (2015) with treatment of calycosin on SKOV3 ovarian cancer cells, which induced apoptosis and acted as anti-growth activity by increasing the Bax/Bcl2 ratio with up-regulation of caspase protein expression. The treatment of cisplatin on various ovarian cancer lines like A2780, CP70 and C30 the data revealed that the cisplatin induces apoptosis in all the cells but through caspase-3 dependent pathways in CP70 & C30 by activating Bax expression, where as in A2780 it was absent (Karen and Turchi 1999).
For the initiation of TNF promoted cell death through extrinsic pathways the caspase-8 very much essential. The activated caspase-8 responsible to cleave caspase-3, caspase-7 and NF-kB to trigger apoptosis hence the caspase-3, which get activated by extrinsic and intrinsic pathway play a key role in executing apoptosis (Carrington et al., 2006; Ghavami et al., 2009; Porter et al., 1999).

The role of phytol in regulating the cell cycle was studied on ovarian carcinoma cells exhibited the IC$_{50}$ concentration at 160 µg/mL and 320 µg/mL and shown the moderate action on cell cycle arrest at the G$_2$M phase and SubG$_0$ phase leading to apoptosis.

The study of Curcumin on ovarian cancer cell has arrested cell cycle at G$_2$/M phase (Weir et al., 2007). In the present investigation the phytol inhibited the proliferation of ovarian cancer cells at G$_2$/M and SubG$_0$ level initiating the apoptosis due to the role of activated CASPASE-3 and CASPASE-8.

Alcoholic extract of *Ganoderma lucidum* treated to the MCF7 cell line revealed that up-regulation of Bax protein expression and initiation of apoptosis indicating the anti-proliferation effect of the extract by arresting cell cycle at G$_1$ phase due to down regulation of E2F & cdk4 and up regulation of p21 (Hu et al., 2002).

The effect of phytol on lung cancer cells (A549) indicated the activation of apoptotic genes caspase 9 & 3 expression at IC$_{50}$ value 16.97±2.31 µM causing intrinsic pathway of apoptosis (Thakor et al., 2017)

In this present investigation the phytol has activated BRCA-1, BRCA-2, CASPASE-3 and CASPASE-8 gene in SKOV-3 cells and arrested cell cycle by increase of 1.08-1.57 fold of BRCA1 gene expression at the dose of 160-320 µg/mL.
treatment. Whereas BRCA2 gene increased from 1.02-1.20 fold expression compared with non-treated cells. However CASAPSE-3 has increased gene expression from 1.15-1.31 fold regulation and CASPASE-8 activation by increase in gene expression from 1.07-1.50 fold through extrinsic pathway.

BRCA1 fold regulation increased from 1-1.80 and BRCA2 were increased from 1-2 fold regulation of gene expression in treated SKOV3 cells by treatment of α-spinasterol isolated from *Ganoderma resinaceum* (Sedky et al., 2017).

The DNA repair and transcriptional regulation is controlled by BRCA-1 and BRCA-2 genes by producing multifunctional proteins and also involved in apoptosis (King et al., 2003).

Based on the above experimental results obtained in this study revealed that phytol play a key role in inhibiting cancer cell proliferation and induction of apoptosis, it is observed in ovarian cancer SKOV-3 cell lines. This might be due to activation of caspase and Bcl2 family protein with a cascade of events, thus the phytol act as potential agent to cure ovarian cancer.