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Anticancer, Antimicrobial and Phytochemical Properties of *Catharanthus roseus* (L.)

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Abstract
The tropical plant Madagaskar Periwinkle (*Catharanthus roseus*) (L.) is an important medicinal plant of family Apocynaceae. The present investigation focused on evaluation of the antimicrobial, anticancer and phytochemical properties of the floral and leaf extract of *Catharanthus roseus* (L.). The plant extracts showed the presence of phytochemicals like flavonoids, phenols, tannins and saponins. Evaluation of the antimicrobial effect *on Escherichia coli* and *Staphylococcus aureus* revealed its potent antimicrobial properties. The In vitro antitumor activity of different extracts of *Catharanthus roseus* was evaluated by the MTT assay using THP-1 leukemic cell lines and Hela cell lines. The floral and leaf extracts demonstrated potential *In vitro* cytotoxic activity against Hela and THP-1 cell lines. Thus, it can be concluded that the plant *Catharanthus roseus* has immense potential for applications in pharmaceutical industry and medicine due to its phytoconstituents, antibacterial and anticancer properties.
Catharanthusroseues. Qualitative analysis of phytochemical screening of both the aqueous and methanolic extract reveals the presence of alkaloids, phenol, saponins, phytosteriods, flavoniods, tannin, quinine, fats and oil. Further the presence of phytochemicals was detected by TLC and paper chromatography which is the standard techniques of separating organic compounds. Antimicrobial activity was done for both methanolic and aqueous plant extracts. The study reveals that the flower extract shows highest antibacterial activity against *Staphylococcus aureus* and *E. coli*. More than 3000 plants species that have reportedly been used in the treatment of cancer and other disease. Plant derived compound have played an important role in the development of several useful anticancer agent. It is significant that 60% of currently used anticancerousagents are derived from natural source. *Catharanthusroseues* is cultivated mainly for its alkaloids, which are having anticancer activity.

Keywords
Catharanthus roseus, phytochemical, anticancer, antimicrobial, medicine, pharmaceutical.

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INTRODUCTION

Plants found in nature have been known to contain medicinal properties since many years. The medicinal plants have secondary metabolites and phytochemicals that have immense application in pharmaceutical industries. These plants contain Phytoconstituents that are bioactives and are causal for different activities such as anticancer, antioxidant and antimicrobial (Irulandi et al., 2016; Hossain and Nagooru, 2011; Suresh and Nagarajan, 2009). Phenolics andflavanoids have antioxidative, anticarcinogenic, antimicrobial activities (Saidu et al., 2012; Sasikumar et al., 2010; Liu et al., 2008). These phtoconstituent are known as potent anti-oxidant agents. Antioxidants are agents which scavenge free radicals and prevent damage caused by reactive oxygen species (ROS). Several medicinal plants have been shown to exhibit potential antioxidant activity due to presence of various phytochemicals, e.g. flavonoids, phenolics, tannins, etc. Plants that contain such phyto-constituents and antioxidants often demonstrate potent anticancer properties. These plants can play an important role as alternative medicine for the treatment of cancer with minimum side effects.

Catharanthus roseus (Linn) is such a perennial herbaceous sub-shrub that belongs to the family *Apocynaceae*. It is commonly called as Madagascar periwinkle, and regional Indian name is sadabahar (in Hindi) or sadafuli (in Marathi) (Rasool et al., 2007). It is well known for its medicinal and pharmaceutically important properties like antibacterial, antifungal and antiviral activities (Jaleel et al., 2007). The leaves of the plant have been reported to contain more than 130 different types of alkaloids (Pereira et al., 2010). In the present investigation, the antimicrobial, anticancer and phytochemical properties of the floral and leaf extract of *Catharanthus roseus* (L.) were studied.

MATERIALS AND METHODS

Plant material and preparation of extract: Fresh leaves and flowers of *Catharanthus roseus* (L.) were collected from S.P.Pune University campus, Pune. The leaves and flowers were washed twice with distilled water, dried and powdered. The plant extract was prepared by adding 100 g of powder of plant material in 450 ml of methanol for methanolic extract and 450 ml distilled water for aqueous extract and boiling in Soxhlet apparatus. The extract was cooled and filtered through Whatman no. 1 filter paper and stored at 4°C in the dark till further use.

Qualitative phytochemical analysis of *Catharanthus roseus* (L.) extract:

Preliminary phytochemical analysis of methanolic and aqueous extracts of flowers and leaves of *Catharanthus roseus* (L.) was done qualitatively to detect the presence of various phytoconstituents. The tests were performed to detect phytochemicals viz. Tannins ($FeCl_3$ test), Alkaloid (Dragendorff's test), Reducing sugars (Fehling's test), Protein (ninhydrin test), Steroids and triterpenoids (Salkowski test) and Saponin (froth test) using methods described previously (Kokate, 2000; Harborne, 1999; Edeoga et al., 2005; Harbone, 1973; Yadav et al., 2014 and Gopinath et al., 2012).

Estimation of Total Phenolic Content of *Catharanthus roseus* (L.) extract: The total phenolic content in various extracts *Catharanthus roseus* (L.) extract was determined by Folin-Ciocalteu reagent method with some modifications (Lister and Wilson, 2001). 10 µl of plant extract was added to 490 µl distilled water to which 2.5 ml Folin- Ciocalteau reagent (SRL) and 2 ml of 7.5% Na_2CO_3 was added. The reaction mixture was incubated for 90 min at room temperature and absorbance was recorded at 750 nm. Gallic acid was used as standard. The results were expressed in mg gallic acid equivalents (GAE) per milligram of dry weight of the plant.

Determination of total flavonoids of *Catharanthus roseus* (L.) extract: Total Flavonoid content in various extracts was determined as described previously (Zhishen et al., 1999). 50 µl of plant extract was added to 4950 µl Distilled water and was mixed with 0.3 ml of 5 % $NaNO_2$. This was incubated for 5 min at room temperature and 0.3 ml of 10 % $AlCl_3$ was added to the mixture. After 6 min of incubation 2 ml of 1M NaOH was added to the mixture followed by addition of 2.4 ml distilled water. Absorbance was recorded at 510 nm. Querscetin was used as standard. The results were expressed in expressed in Querscetin equivalents (QE) per milligram of dry weight of the plant.

Evaluation of antibacterial activity *Catharanthus roseus* (L.) extract: The antibacterial activity of *Catharanthus roseus* (L.) extractwas tested against *Escherichia coli* and *Staphylococcus aureus.* The antibacterial activity was assessed by seeding 0.1 ml of test bacterial culture (Optical density at 600 nm = 0.5) on Nutrient agar plates (Hi Media, India). 6mm wells were made on agar surface with cork borer to which 40 µl of each methanolic and aqueos extract of leaves and flowers was added. Plates were

incubated at 37°C for 24 hrs. and zone of inhibition was measured.

Anticancer activity of *Catharanthus roseus* (L.) extract against THP-1 and HeLa cell line: The cytotoxic activity of extracts of *Catharanthus roseus* (L.) on THP-1 and HeLa cell lines was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2-5-diphenyltetrazoliumbromide) assay (Artasasta et al., 2017). The cell culture was purchased from National Centre for Cell Sciences, Pune. They were cultured in RPMI and DMEM with 10% Fetal Calf Serum (FCS). THP-1 (Acute Myeloid Leukemia cell line) cells and HeLa cells were seeded in 96-well plates at a density of 1.0×10^4cells/well. It was then incubated overnight at 37°C in a 5% CO_2 humidified environment. The different extracts of *Catharanthus roseus* (L.) were added to wells. After incubation for desired period of time at 37°C in humidified incubator, cell viability was assessed by MTT assay. The cells were incubated with MTT for 4 hrs, they were centrifuged, MTT was removed and 100 µl DMSO containing 25 µl Glycine buffer was added to each well. Absorbance was measured at 595 nm (Permanasari et al., 2016).

RESULTS AND DISCUSSION

The floral and leaf extracts of *Catharanthus roseus* (L.) were explored for the presence of phytochemicals. The results showed that leaves *Catharanthus roseus* (L.) had the presence of alkaloids, phenols, tannins and saponins. The phytoconstituent steroids was absent in all of the plant extracts. The phytochemical constituents of the plants investigated are summarized in Table 1

Table 1: Qualitative phytochemical screening of *Catharanthus roseus* (L.) (Legend: + present, - absent)

Phytochemical	*Catharanthus roseus* (L.) Flower		*Catharanthus roseus* (L.) Leaves	
	Aqueous	Methanol	Aqueous	Methanol
Alkaloid	+	–	+	–
Carbohydrate and glycoside	+	+	+	+
Protein	–	+	+	–
Saponin	+	+	+	+
Steroid	–	–	–	-
Phenol	+	+	+	+
Tannin	+	+	+	+

The phytochemical constituents like alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms (Bonjar et al., 2004). Hence, the different extracts of *Catharanthus roseus* (L.) were tested against Gram positive and Gram-negative bacteria including *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial activity was determined agar well diffusion method and the zone of inhibition was recorded. The extracts showed effective antibacterial activity against the test organisms as in Table 2.

Table 2: Antibacterial activity of extracts of *Catharanthus roseus* (L.)

Test organisms	Diameter of zone of inhibition (mm)			
	***Catharanthus roseus* (L.) Flower**		***Catharanthus roseus* (L.) Leaves**	
	Aqueous extract	**Methanolic extract**	**Aqueous extract**	**Methanol extract**
Escherichia coli	-	9	-	2
Staphylococcus aureus	2	5	-	4

The results demonstrated highest antimicrobial activity in methanolic extract of flower. While the aqueous extract of leaves of *Catharanthus roseus* (L.) did not show any antimicrobial activity. Currently, chemotherapy is the main treatment for cancer, and it induces serious side effects on patients. Extensive studies seeking new active plant extracts that can be used in the treatment of cancer have been carried out in the search for drug candidates that have high efficacy and safety (Ni et al., 2010). The anticancer

potentials of *Catharanthus roseus* (L.) Flower and leaf extract were studied and the results are presented in Table 3.

Table 3: Anticancer activity of *Catharanthus roseus* (L.) extract against THP-1 and HeLa cell line

Cell Line	Percentage survival			
	Catharanthus roseus (L.) Flower		*Catharanthus roseus* (L.) Leaves	
	Aqueous extract	Methanolic extract	Aqueous extract	Methanol extract
HeLa	82	99.96	85.05	72.65
THP-1	86.68	79.83	62.77	67.61

The evaluation of cytotoxic activity is based on quantification of purple colored formazan, which is formed by the reduction of MTT [3-(4, 5-dimethylthiazole-2-yl))-2, 5-diphenyl tetrazolium bromide]. The reduction of MTT is proportional to the number of active mitochondria in the living cells. The cytotoxic activity of *Catharanthus roseus* (L.) Flower and leaf extract was assessed against THP-1 leukemia cell line and Hela cell line. The leaf aquoes and methanolic extract showed maximum growth inhibition in comparison to the floral extracts against both the THP-1 and Hela cell lines. The methanolic extract of *Catharanthus roseus* (L.) flower showed least activity. Drug resistance in microorganisms is an emergent phenomenon that poses challenges to public health and treatment. Besides chemotherapy in cancer have lots of side effects. Hence, the plant extracts of *Catharanthus roseus* (L.) can be explored for its applications in medicine for combating bacteria and inhibition of cancer cells. However, more extensive studies related to toxicity, dose dependence and purification of metabolites need to be undertaken for future applications in medicine and pharmaceutical industry.

CONCLUSION

The present study demonstrates that the presence of various phytochemicals in the different solvent extracts of *Catharanthus roseus* L. Antimicrobial activity of plant extract has been shown it has a broad spectrum of activity. The floral and leaf extracts also demonstrated potential *In vitro* cytotoxic activity against Hela and THP-1 cell lines. Thus it can be concluded that the plant *Catharanthus roseus* has immense potential for applications in pharmaceutical industry and medicine due to its phytoconstituents, antibacterial and anticancer properties.

In the present study phytochemical (qualitative and quantitative), chromatography (thin layer chromatography, paper chromatography) antimicrobial and anticancer cell line study have been done using *Catharanthus roseus* plant water and methanol extract among the 2-extract tried methanol extract of leaf and flower was found to best extract for all the studies.

Methanol extract of leaf and flower shows the presence of alkaloids, carbohydrates and glycosides, proteins, quinine, phenol and tannin, fat and oil. Aqueous extract of leaf and methanol extract show the presence of protein, saponin, phenol and tannin, fat and oil.

Methanol and aqueous extract shows the absence of phylobatanin, steroids, mucilages and gum. These extract further purified using silica gel coated TLC plate and paper chromatography.TLC study reveals the presence of compound in the plant extract and its RF value calculated. Paper chromatography reveals the presence of carotene, chlorophyll a, chlorophyll b, xanthophylls. The result of maximum antibacterial activity was identified with methanol extract of leaf and flower against *E.coli* and *S.aureus* and this antimicrobial activity of methanolic extract might be due to presence of unique phytochemical constituents. The He La cell line and THP cell line cytotoxins study was also conducted and the % survival rate of cell was calculated of each extract in given table with graph. Methanol extract of leaf and flower shows more cytotoxic effect on cancerous cell line.

DISCUSSION

Catharanthus roseus medicinal plant is the most exclusive source of life saving drugs for majority of world's population. They continue to be an important therapeutic aid for alleviating the ailments of human mankind's. India has a rich and diverse flora of flowering medicinal plants. this plant plays a vital role in human health care, about 80% of the

world population role on the use of traditional medicine, concomitantly based on plant materials.

The phytochemical screening for methanol aqueous plant extract of *Catharanthus roseus* shows presence of alkaloids, phytosterols, phenolic compound, flavonoids compounds, saponin, tannin, fats and oil, glycoside, carbohydrate and terpeniods. The test plant parts show the absence of phylobatanins and steroids. These compounds were further separated by the paper chromatography which shows the presence of carotene, xanthophyl, chlorophyll a, chlorophyll b, xanthophylls. TLC plates with purple to violet bands were observer under UV. It indicates presence of flavonoids. The RF value were calculated for all the methanol and aqueous plant extract. TLC plate showed bands only for methanol leaf and flower extract. The results of the maximum antibacterial activity was identified with and methanolic leaf and flower extract of *C. roseus* against *S. auerus*and *E.coli* the antimicrobial activity of the methanol extract might be due do the presence of the unique phytochemical constituents. The plant of *C. roseus* has a very great medicinal value. The antimicrobial activity found in this present study may be attributed to the presence of secondary metabolites of varies chemical types present in the plant material either individually. The discovery of apotent remedy from plant origin will be great advancement in microbial infection therapies. The two bacterial strains *S. aureus* and *E. coli*used in this experiment are responsible for human diseases such ascholecystitis, urinary infection, skin infections etc. However, these human pathogenic strains were significantly inhibited by the methanolic leaf extracts of the medicinal plants. The discovery of a potent remedy from plant origin will be great advancement in microbial infection therapies. Leaf and flower extract of C*atharanthus roseus* shows a toxic effect on cancerous cell line (HELA cell line and THP cell line) Hence, this study holds importance in using medicinal plants as an alternative source for treating cancer and various disease.

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Phytochemical analysis of *Catharanthus roseus*

Phytochemical analysis of***Catharanthus roseus*** (flower and methanol) was carried out in aqueous, methanol extract and results are shown below

Fig 1: phytochemical test of leaf methanolic and aqueous extract

Extracts and tests	Flower		Leaf	
	F.M	F.AQ	L.M	L.AQ
1] Alkaloid	+	_	+	_
2]Carbohydrate and glycoside	++	+	++	+
3]Protein	_	++	++	_
4]Saponin	++	++	++	++
5]Phylobatanin	_	_	_	_
6]Steroid	_	_	_	++
7]Quinine	_	_	+	+
8]Mucilage and gum	_	_	_	_
9]phenol and tannin	+	+	+	+
10]fat and oil	+	+	+	+

++ = Presence of high constituents - = Absence of high constituent
+= Presence of low constituent

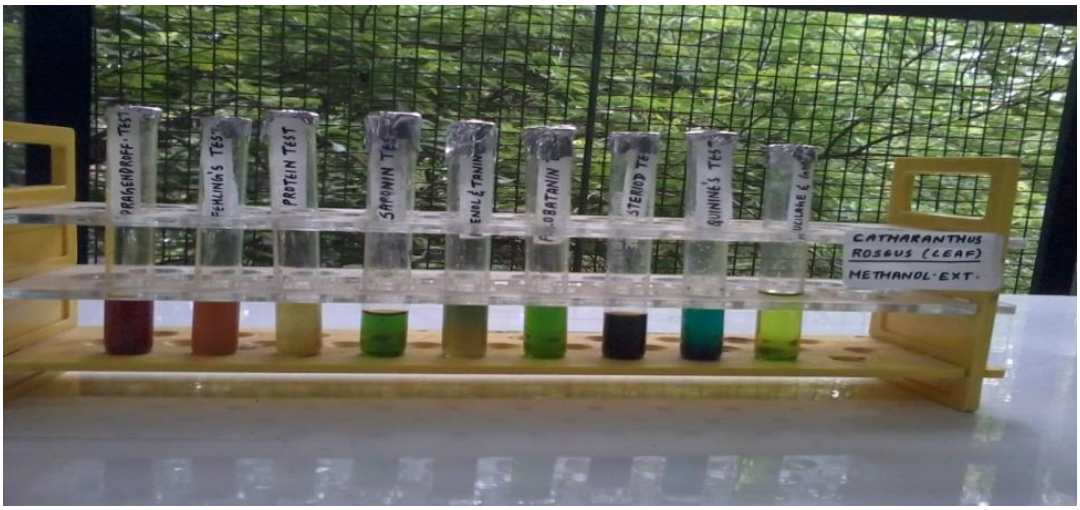


Fig no: - Phytochemical test of leaf methanolic extract.

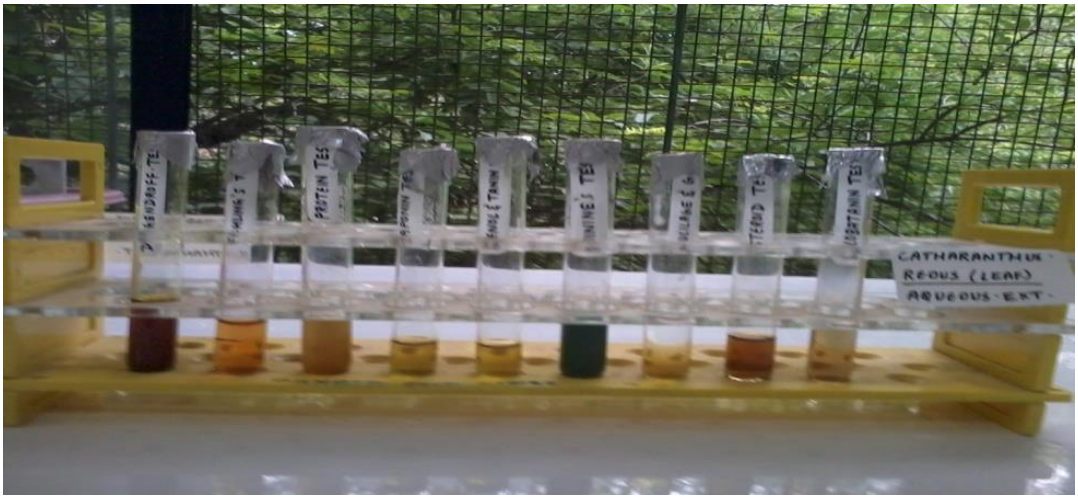
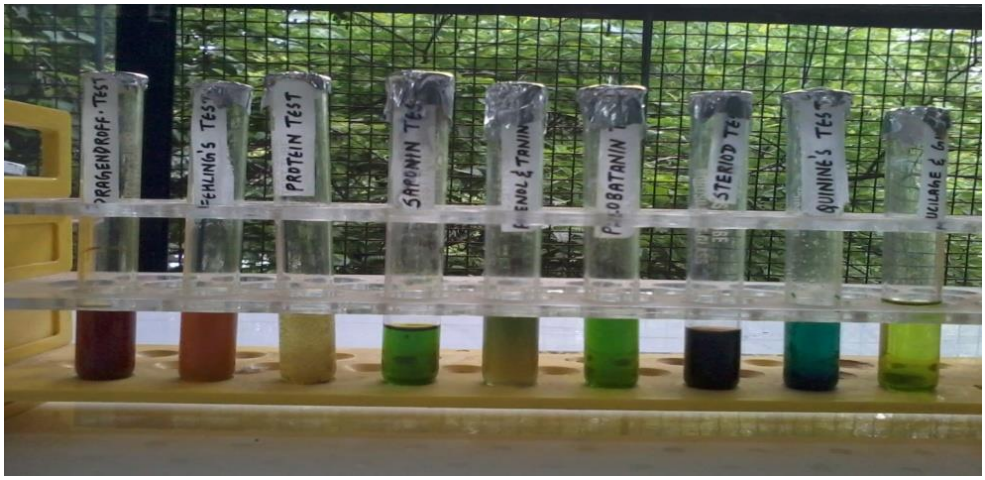


Fig no: -phytochemical test of leaf aqueous extract.

Phytochemical test of flower methanol extract

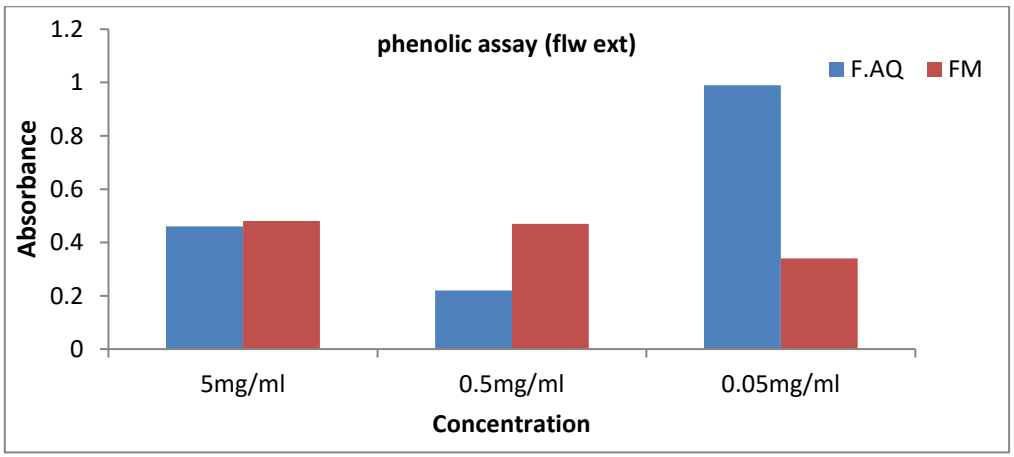


Phytochemical test of flower aqueous extract

Total phenolic content

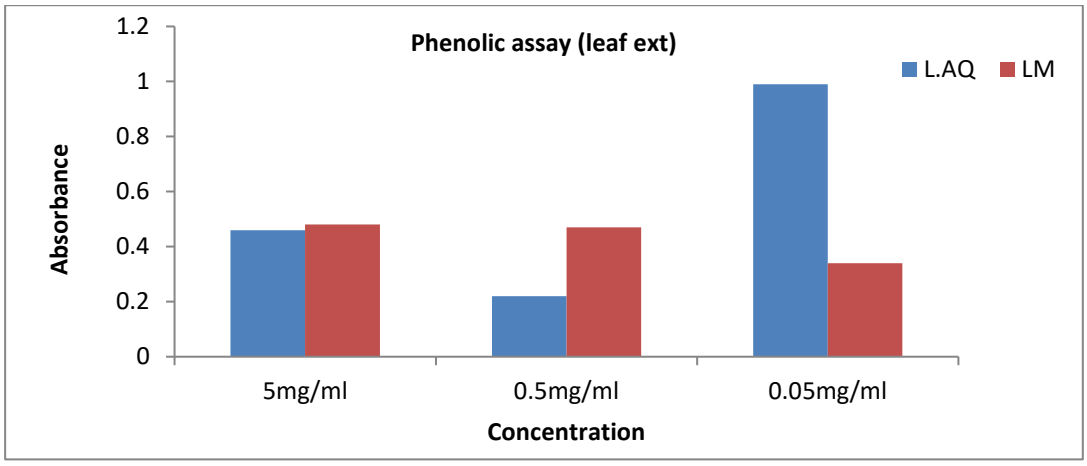
Flower extract at different concentration

Phenolic content		
Concentrations(mg/ml)	**D/W extract**	**Methanolic extract**
5	**0.46**	**0.48**
0.5	**0.22**	**0.47**
0.05	**0.99**	**0.34**



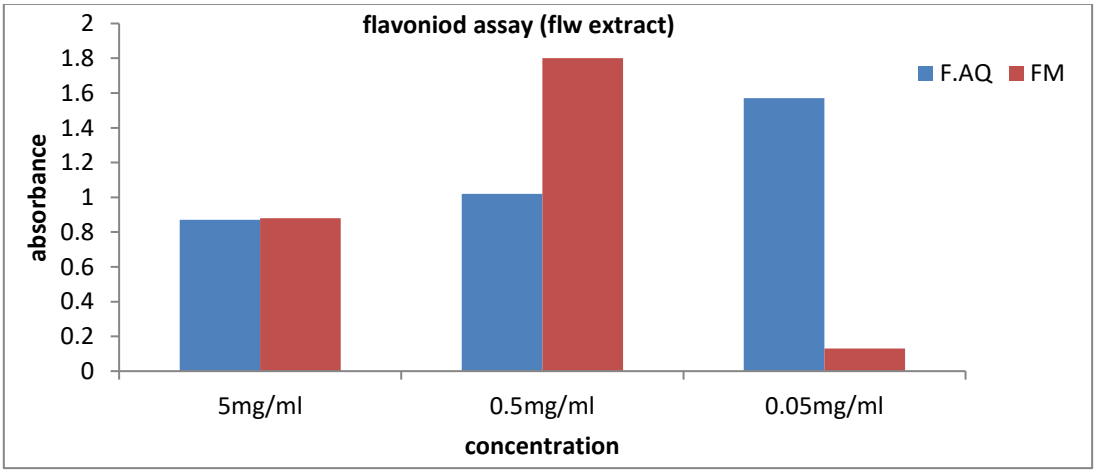
Total phenolic content

Leaf extract at different concentration		
phenolic content		
Concentration (mg/ml)	**D/W Extract**	**Methanolic extract**
5	**0.25**	**0.27**
0.5	**0.93**	**0.30**
0.05	**0.46**	**0.34**



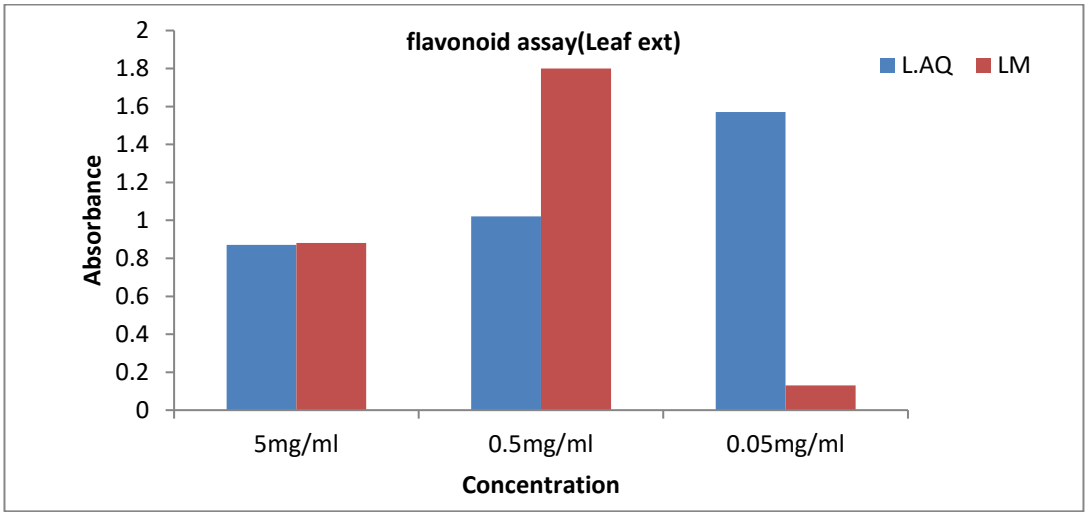
Total Flavonoid content

Flower extract at different concentration		
Flavonoids content		
Concentrations (mg/ml)	**D/W Extract**	**Methanolic extract**
5	**0.87**	**0.88**
0.5	**1.02**	**1.80**
0.05	**1.54**	**0.13**



Total flavonoid assay

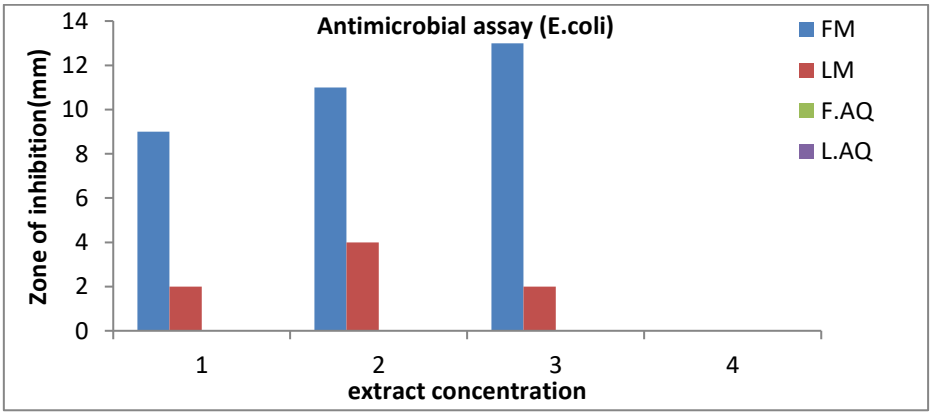
Leaf extract at different concentration		
Flavonoids content		
Concentrations	**D/W Extract**	**Methanolic extract**
5	**0.99**	**0.88**
0.5	**1.23**	**1.01**
0.05	**1.22**	**0.90**

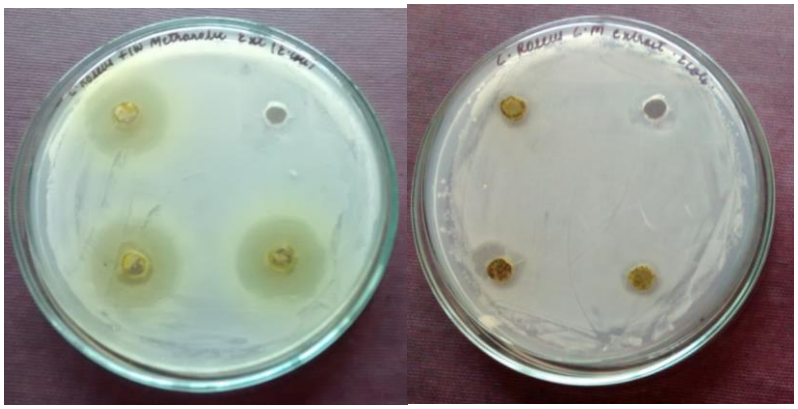


Antimicrobial activity

Antimicrobial activity of Methanolic and Aqueous extract against E.coli

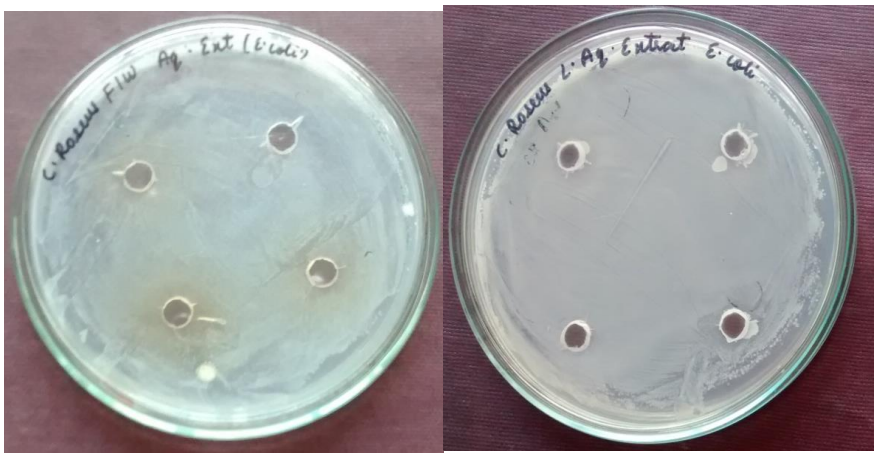
Microbe	***E.coli***			
Concentration	**Flw met ext**	**Leaf met ext**	**FlwAq.ext**	**Leaf aqext**
5mg/ml	**9**	**2**	**-**	**-**
0.5mg/ml	**11**	**4**	**-**	**-**
0.05mg/ml	**13**	**2**	**-**	**-**





Zone of inhibition produced from methanolic extract

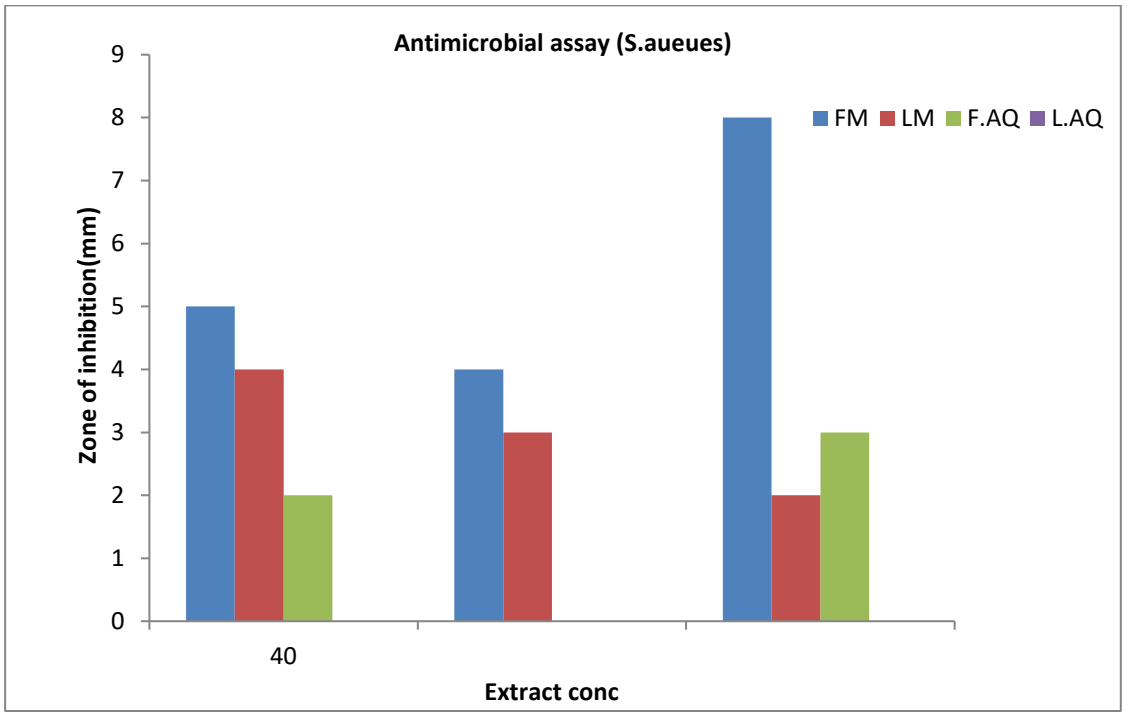
Inhibition of E. coli growth by flower extract at 5,0.5,0.05mg/ml conc.
Inhibition of E. coli growth by leaf extract at 5,0.5,0.05mg/ml conc.

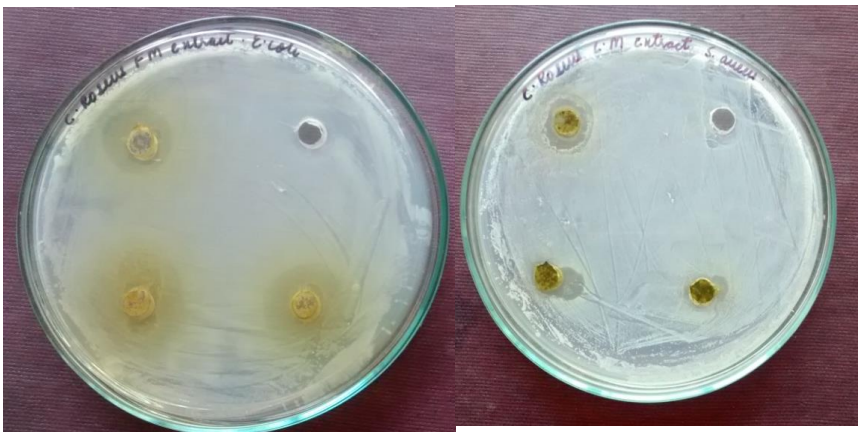


Zone of inhibition produced from aqueous extract
Inhibition of E. coli growth by flower extract at 40,60,80µl concentration.
Inhibition of E.coli growth by leaf extract at 5,0.5,0.05mg/ml conc.

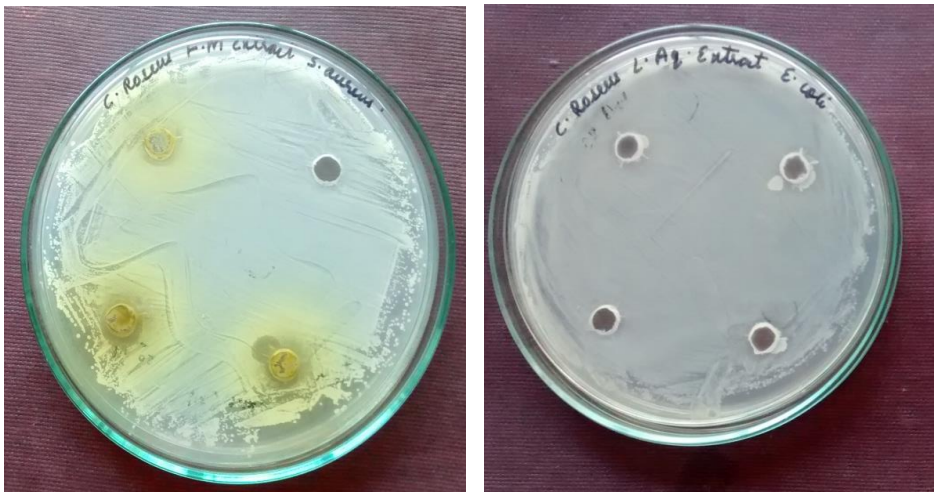
Antimicrobial

Microbe	***Staphylococcus aureus***			
Concentration	**Flw met ext**	**Leaf met ext**	**FlwAq.ext**	**Leaf aqext**
5mg/ml	**5**	**4**	**2**	**-**
0.5mg/ml	**4**	**3**	**-**	**-**
0.05mg/ml	**8**	**2**	**3**	**-**





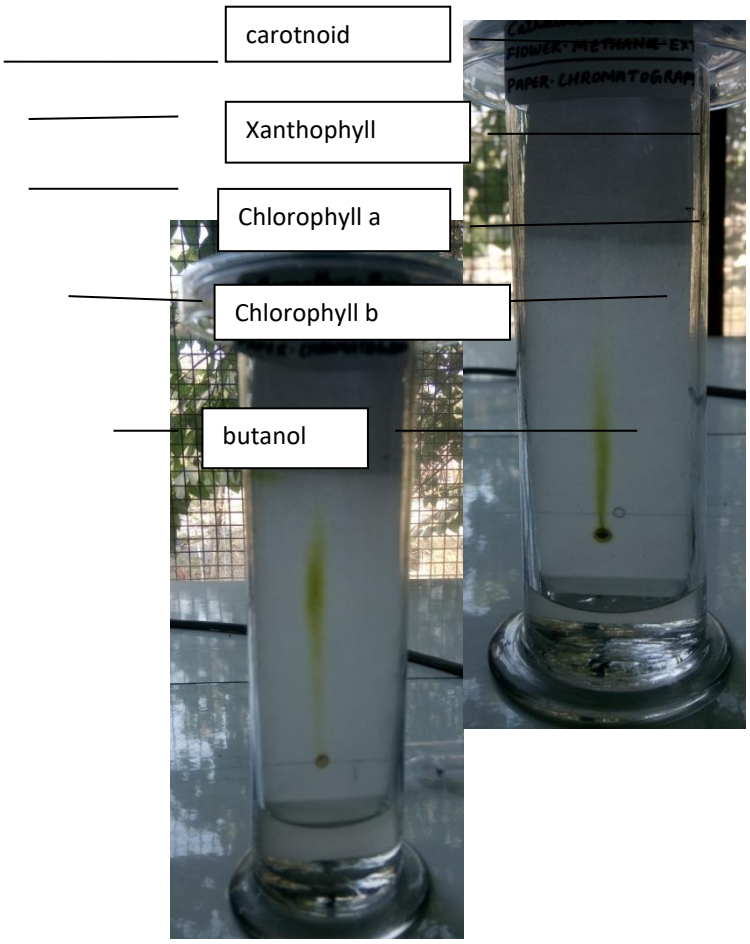
Zone of inhibition produced from methanolic extract
Inhibition of staphylococcus aureus growth by flower extract at 5,0.5,0.05mg/ml concentration.
Inhibition of staphylococcus aureus growth by leaf extract at 5,0.5,0.05mg/ml concentration.



Zone of inhibition produced from aqueous extract
Inhibition of S. aueues growth by flower extract at 40,60,80µl conc.
Inhibition of S.aueuses by leaf extract at 40,60,80µl conc.

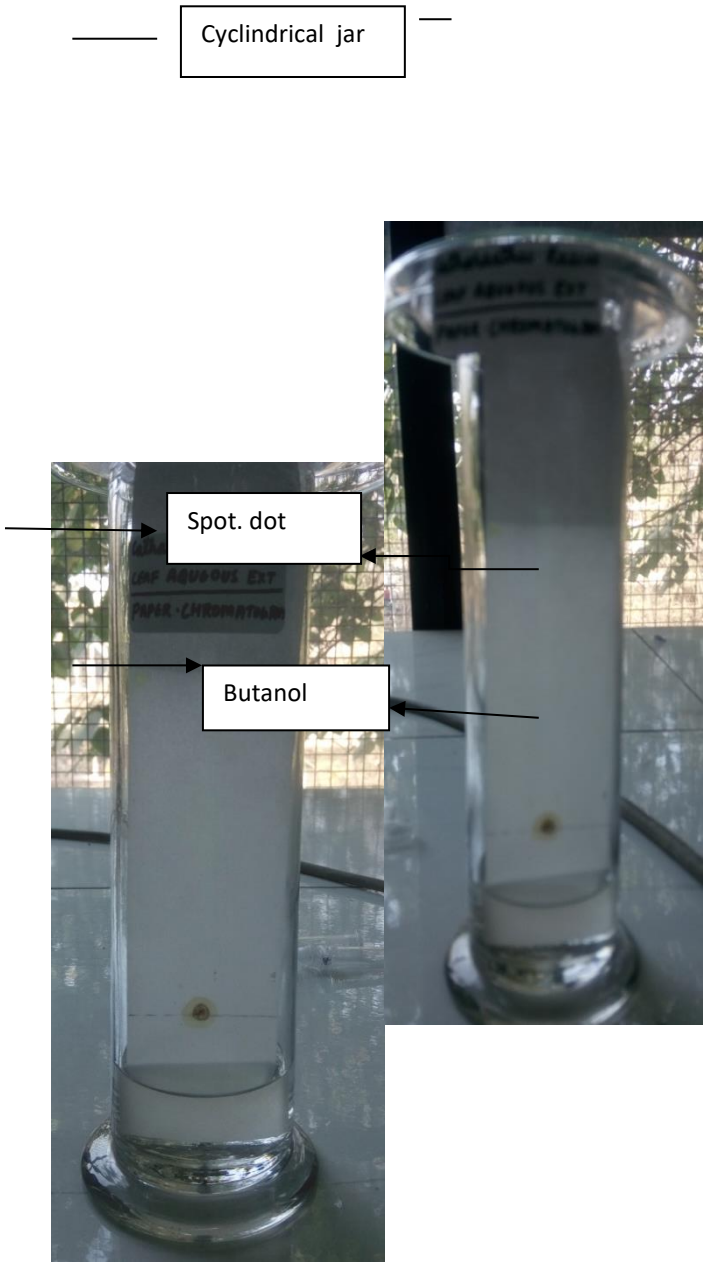
Chromatography
Paper chromatography
Paper chromatography of leaf and flower methanolic extract.

Fig:- leaf methanolic **Fig:-flower methanolic**



Paper chromatography of leaf and flower aqueous extract

Fig:-leaf aq **Fig:-flower aq**


Cyclindrical jar
Spot. dot
Butanol

Thin layer chromatography

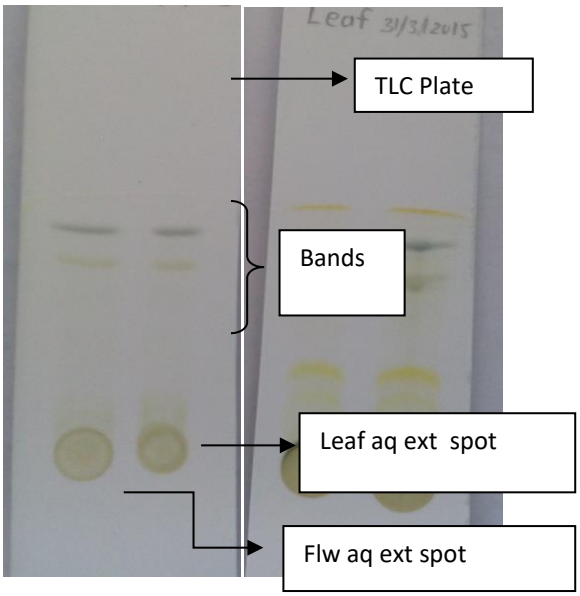


Fig:- TLC of aqueous extract Of flower and leaves

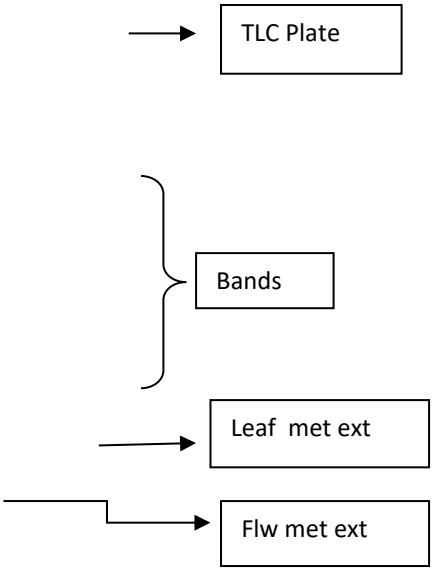


fig :- TLC of methanolic extract of flower and leaves

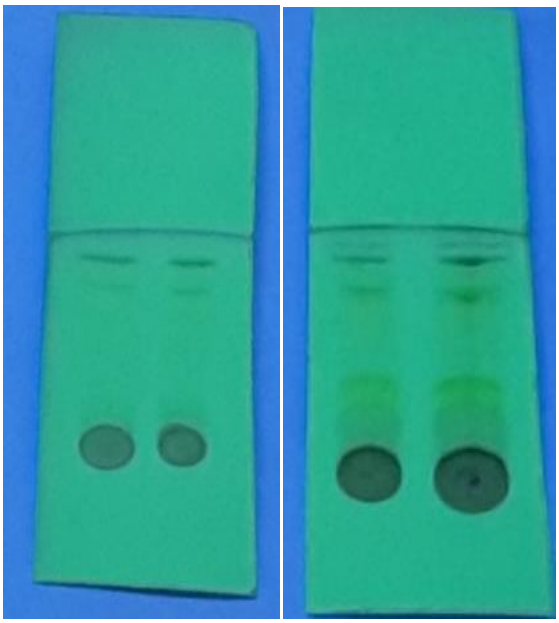


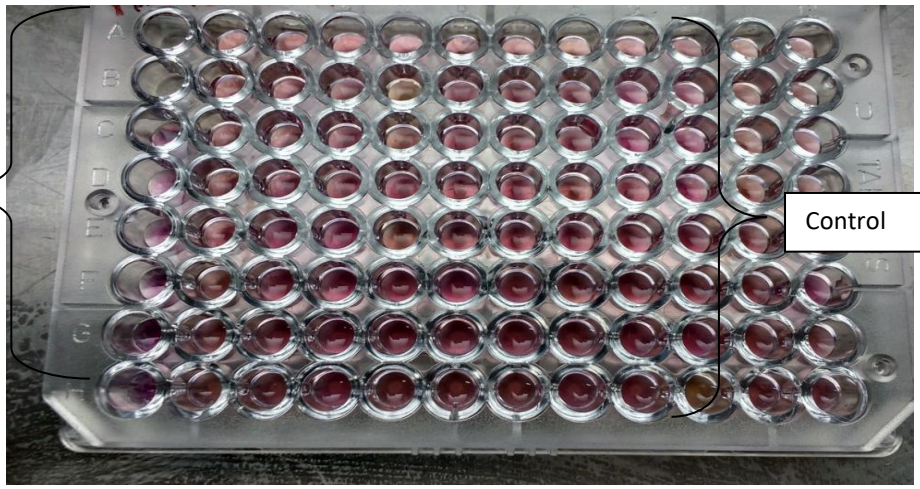
Fig:- TLC plates observe under UV light

6 RF value of TLC bands.

Sr.no	Band	RF value (FM)	RFvalue (LM)	RF value(F.AQ)	RF value (L.AQ)
1.	1.	0.24	0.28	0.76	0.76
2.	2.	0.33	0.37	0.97	0.98
3.	3.	0.68	0.74		
4.	4.	0.89	0.91	No bands were observed.	
5.	5.	0.93	0.92		

MTT Assay
HELA cell line

T-Flask (HELA cell line)



Blank

Control

MTT Assay

Formula: -

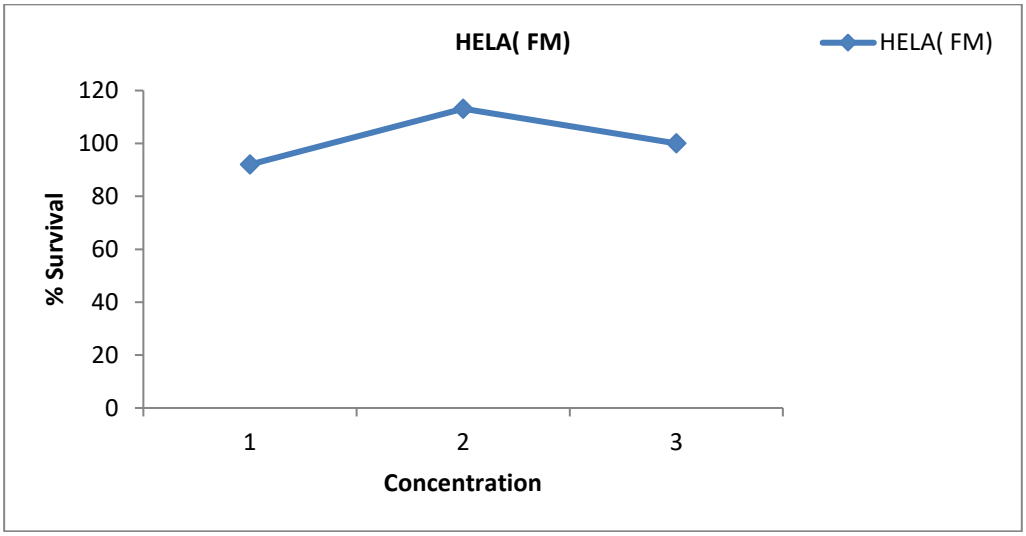
$$\% \text{ of survival} = \frac{\text{ODOFTESTSAMPLE}}{\text{ODOFCONTROL}} * 100$$

96 wells Microtitre plate

CELL LINE	ABS CON
He La	2.5
THP	1.8

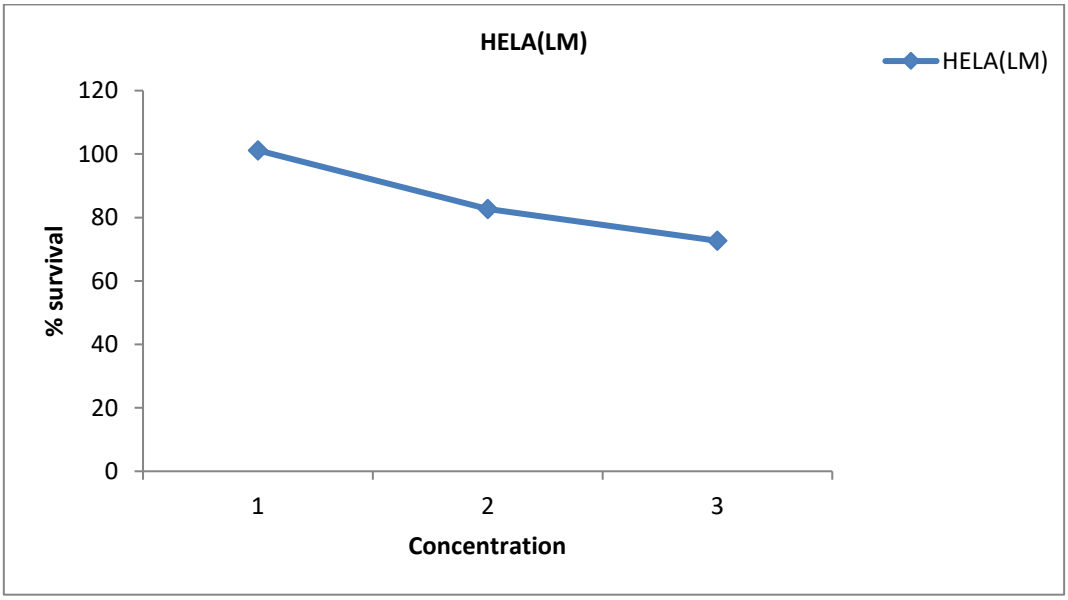
flower methanolic extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	5	2.12	2.53	2.15	2.30	92.02
2.	0.5	2.57	2.79	2.82	2.82	113.13
3.	0.05	2.87	1.59	2.49	2.46	99.96



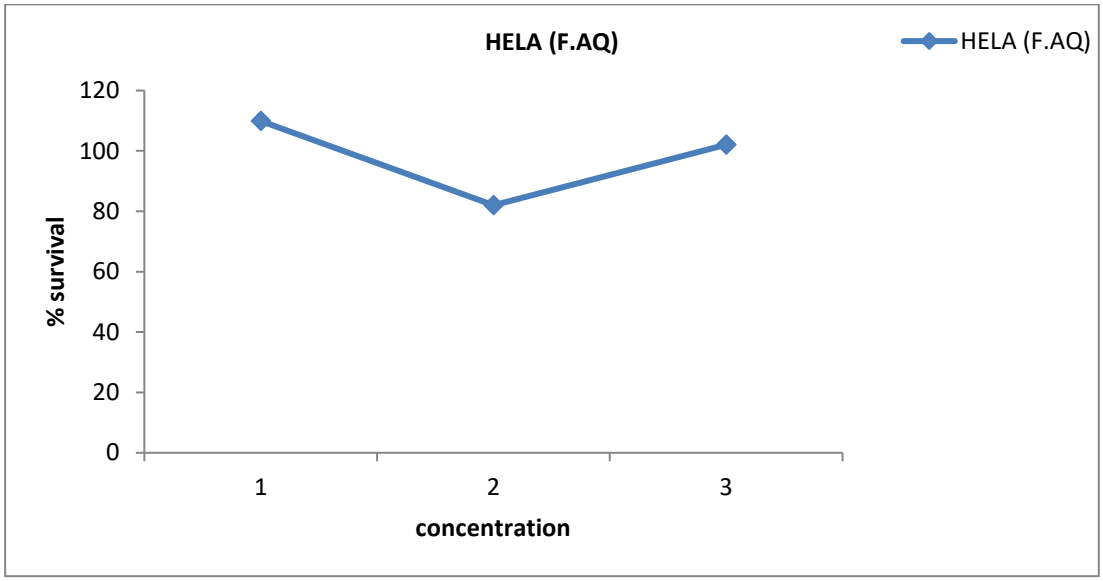
Leaf methanolic extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	5	3.02	1.77	2.79	2.52	101.17
2.	0.5	2.33	1.36	2.50	2.06	82.72
3.	0.05	2.43	1.48	1.52	1.81	72.65



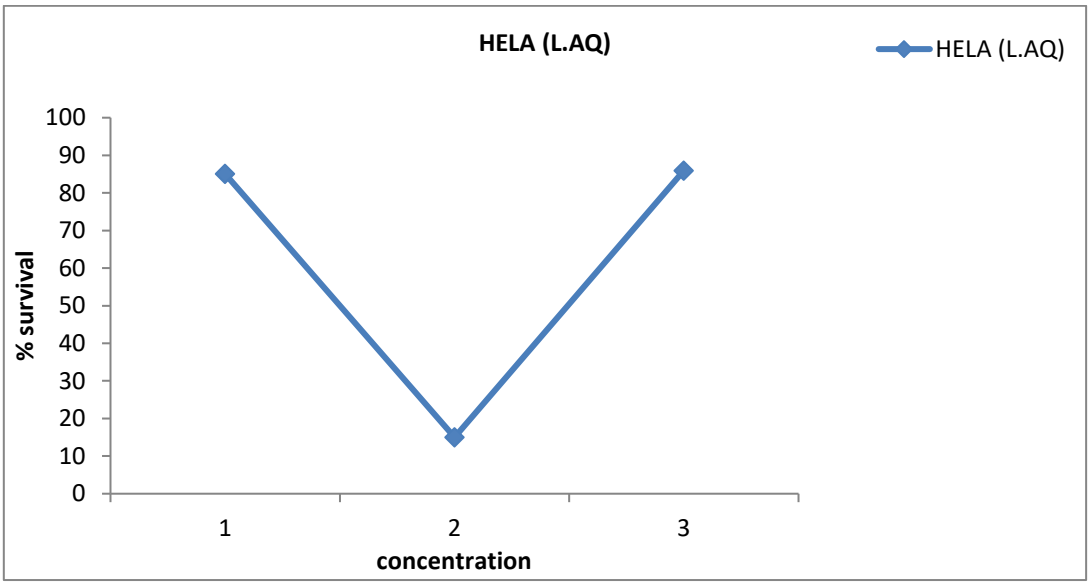
Flower aqueous extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	**5**	**2.66**	**2.57**	**3.00**	**2.74**	**109.90**
2.	**0.5**	**1.06**	**2.50**	**2.58**	**2.05**	**82**
3.	**0.05**	**3.20**	**2.06**	**2.39**	**2.55**	**102..5**



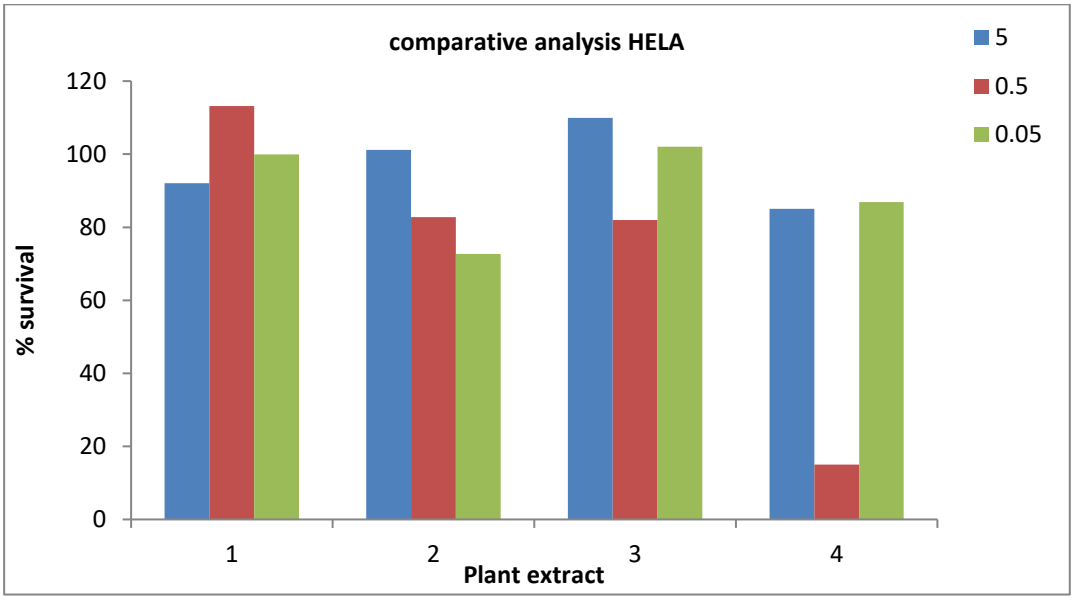
Leaf aqueous extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	5	2.96	1.54	1.86	2.12	85.05
2.	0.5	0.25	0.25	0.61	0.37	15
3.	0.05	1.56	1.64	3.22	2.14	85.92

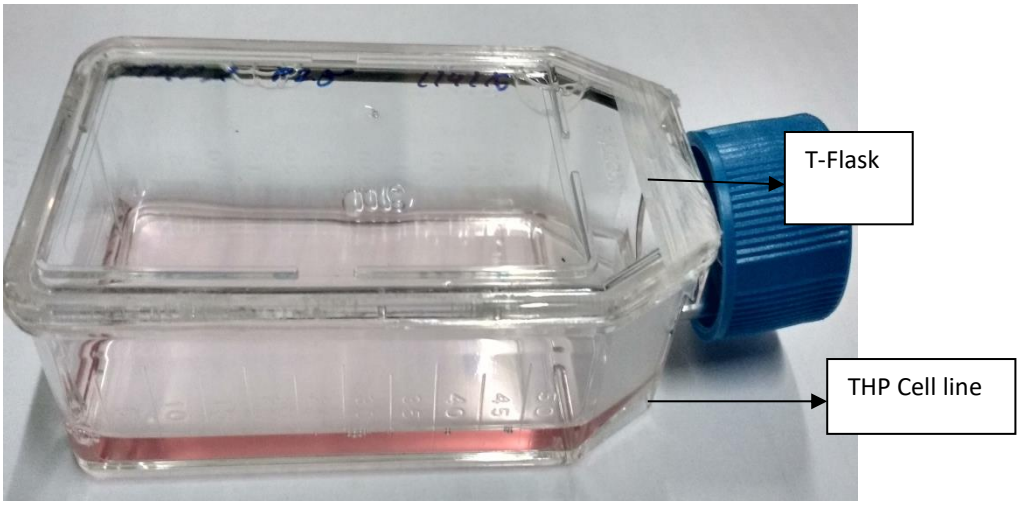


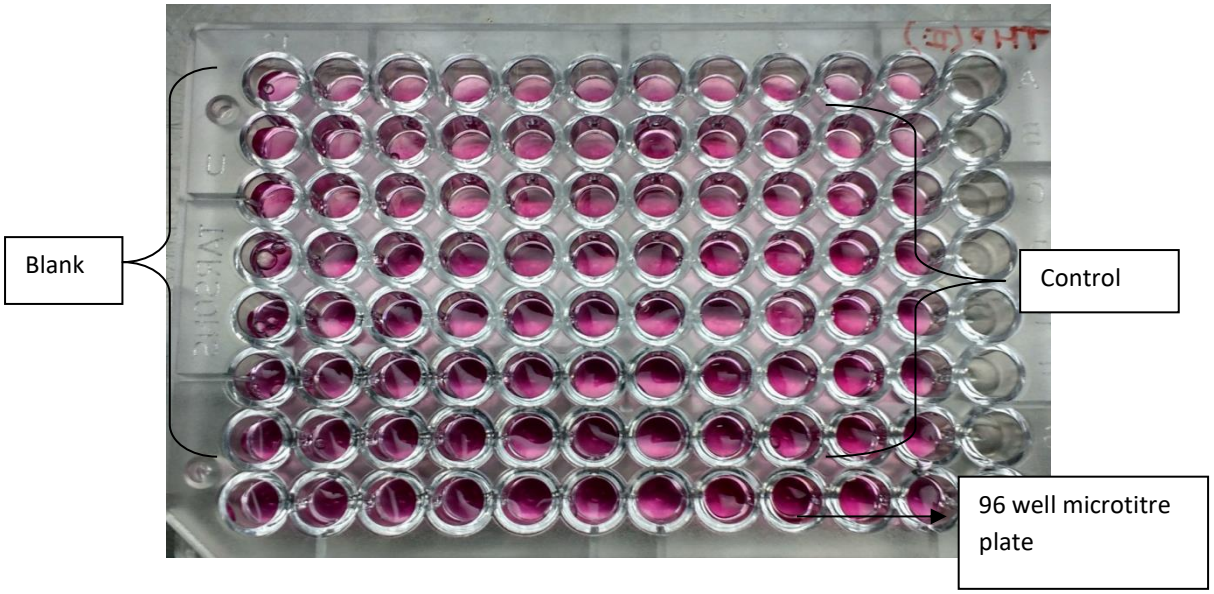
Comparative analysis of methanolic and aqueous extract of flower and leaf on HELA cell line

Concentration mg/ml	% survival			
	FM	LM	F.AQ	L.AQ
5	92.02	101.17	109.96	85.05
0.5	113.13	82.72	82	15
0.05	99.96	72.65	102.05	86.92



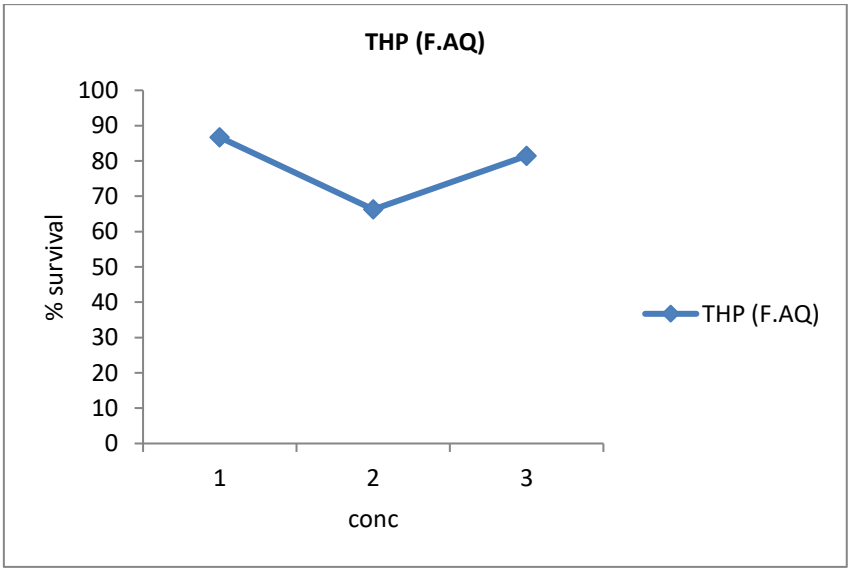
THP cell line





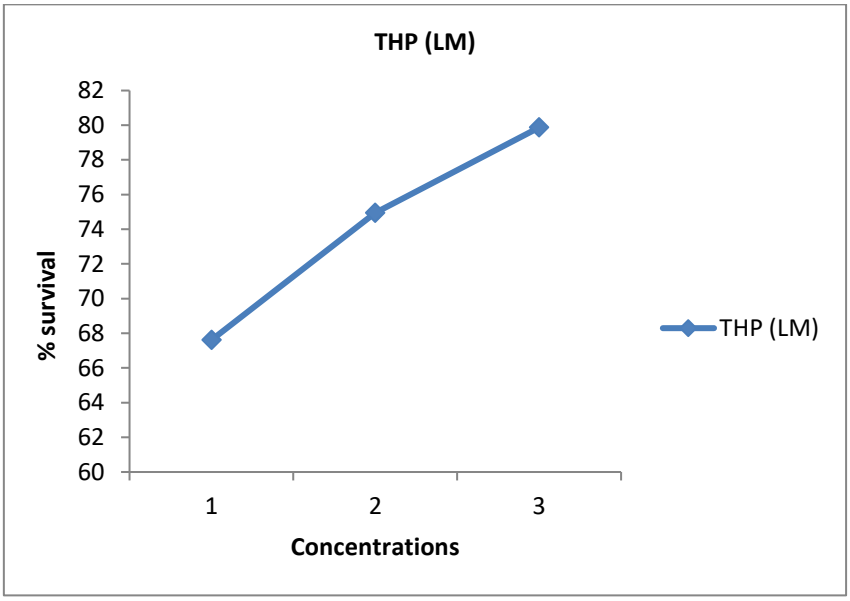
Flower methanolic extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	**5**	0.954	0.982	2.375	1.437	79.83201
2.	**0.5**	1.36	1.047	1.467	1.291333	71.74074
3.	**0.05**	0.818	1.211	1.755	1.261333	70.07407



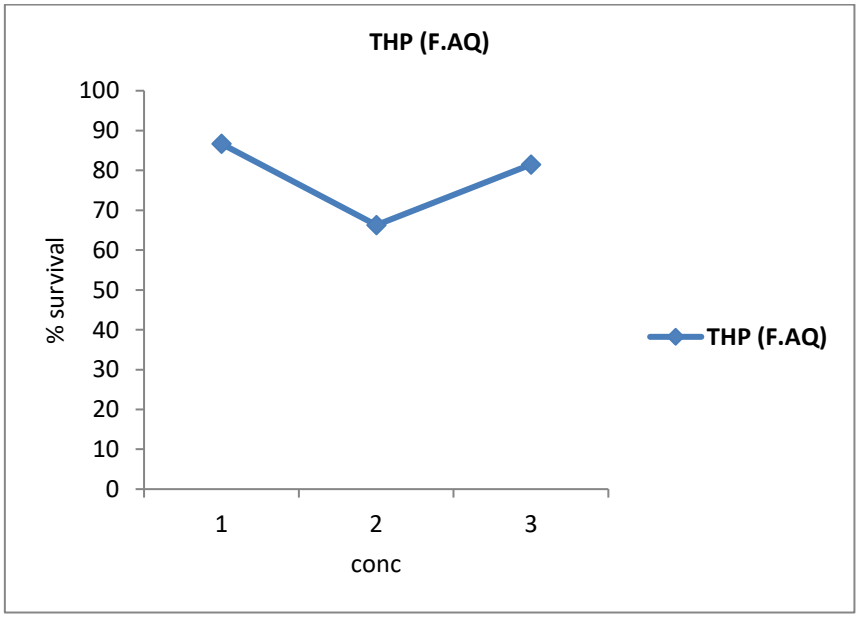
Leaf methanolic extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	**5**	0.097	1.26	1.40	1.21	67.61
2.	**0.5**	1.01	1.35	1.68	1.34	74.94
3.	**0.05**	1.74	1.18	1.38	1.43	79.87



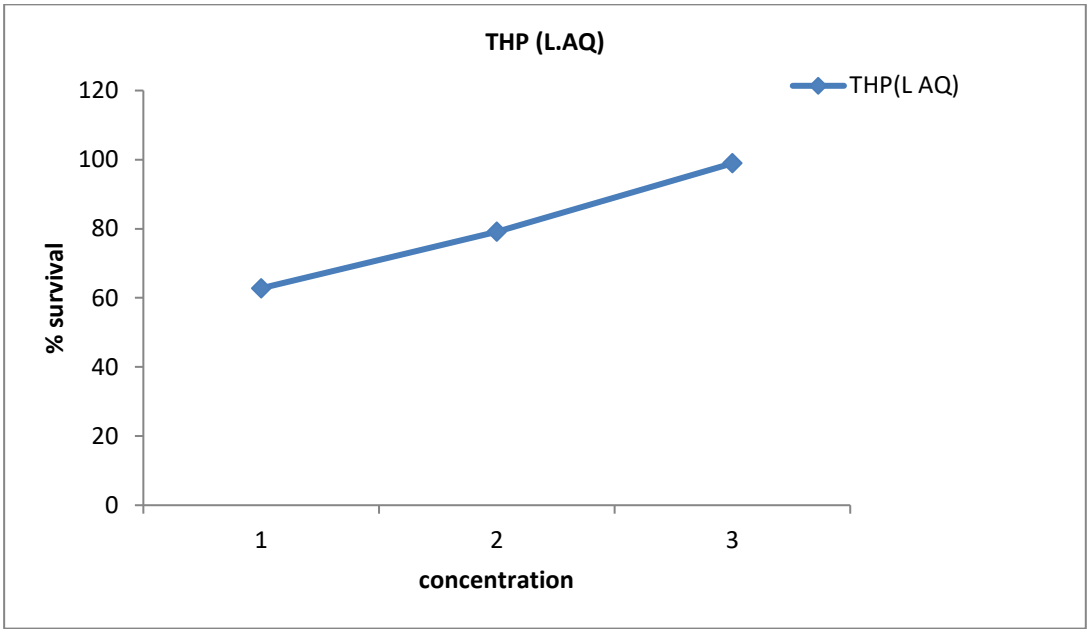
Flower aqueous extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	**5**	1.621	1.505	1.555	1.560333	86.68519
2.	**0.5**	1.191	1.119	1.269	1.193	66.27778
3.	**0.05**	1.228	1.376	1.796	1.466667	81.48148



Leaf aqueous extract

Sr.no	Conc (mg/ml)	HELA cell line absorbance			Avg	% survival
1.	**5**	0.725	1.315	1.35	1.13	62.77778
2.	**0.5**	1.38	1.277	1.615	1.424	79.11111
3.	**0.05**	1.548	1.875	1.92	1.781	98.94444



Comparative analysis of methanolic and aqueous extract of flower and leaf on THP cell line

Concentration mg/ml	% survival			
	FM	LM	F.AQ	L.AQ
5	79.83	67.61	86.68	62.77
0.5	71.74	74.94	66.27	79.11
0.05	70.07	79.87	81.48	98.94