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PRELIMINARY PHYTOCHEMICAL, ANTIBACTERIAL AND *IN VITRO* ANTICANCER ACTIVITY OF METHANOLIC EXTRACTS OF (LEAF AND STEM) *HELIOTROPIUM INDICUM* LINN.

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ABSTRACT

The present study was carried out to determine the preliminary phytochemical and antibacterial activity of methanolic extracts of (leaf and stem) *heliotropium indicum* L. (Family: Boraginaceae). Perliminary phytochemcial tests of in this plant leaf and stem confirm various phytohemical like Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Saponin and carbohydrates. Antibacterial activity against *Bacillus subtilis, Streptococcus mutants, Escherichia coli and Bacillus cereus.* Compare that four microorganisms of methanol extracts of this plant leaves and stem showed antimicrobial activity against rich in *Escherichia coli.* Maximum clear zone of inhibition was found in *Escherichia coli* bacteria. In this study give the result of *Heliotropium indicum* stem and leaf were showed the presence of secondary metabolites like Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Saponin and carbohydrates. And also these are against to the antibacterials (*Bacillus subtilis, Streptococcus mutants, Escherichia coli and Bacillus cereus).* This plant leaf is essential for prevent the Human Breast cancer.

KEYWORDS: Preliminary Phytochemical, Antibacterial, Invitro anticancer, MTT, MCF-7 and *Heliotropium indicum* Linn.

1. INTRODUCTION

Phytochemical screening of various extracts such as aqueous, ethanol, chloroform, acetone and petroleum ether of leaf extracts, revealed the presence of tannins, saponin, phenols,

flavonoids, cardiac glycosides, terpenoids, alkaloids and steroids.[3] These chemicals are present in different parts of plants like stem, leaves, roots, flowers, inflorescence, fruits and seed.[2,6] Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the sources of potential and powerful drugs.[12]

Cancer is one of the deadliest diseases and causes millions of human deaths every year. Estimation showed that approximately 180 million people have died by cancer between 2005 and 2015.[1] Many pharmacognostical and pharmacological investigations are carried out to identify new drugs to develop novel therapeutic agents for the treatment of human cancer to overcome these potential problems with chemotherapeutics.[4,5] Preventing tumor growth must be objective the multiple physiological and biochemical pathways that lead to tumor development. A good correlation exists between antioxidant intakes and reduces cancer risk. Therefore, the antioxidant supplements as part of diet often are used to prevent cancer.[11]

Heliotropium indicum. Linn. Belongs to family Boraginaceae. This family is well marked in their characteristic and not easily confused with any other. A majority of the plant in family are herbs. The genus *Heliotropium* comprises about 250 species and is distributed in tropical, subtropical and warm temperature zones of all continents but only a few species have been systematically investigated.[7] The plant is annual, erect, branched hirsute plant about 15 to 50cm high. The leaves are always opposite or alternate, ovate to oblong- ovate, somewhat hairy, acute or acuminate, base decurrently along the petiole and about 3 to 8cm long. The flowers are calyx green and about 3.5mm in diameter. The fruits are dry 2 to 4 lobed of 2 or 4 nearly free, more or less united nutlets, 4 to 5mm long.[9]

2. MATERIALS AND METHODS

2.1. Collection of plant material

The plants were collected from vennandhur, Namakkal district. They were identified with the help of by Gamble (1935) and ‘The flora of the Tamilnadu, Carnatic India” K.M. Mathew, 1988).

2.2. Preparation of plant powder for extraction

The fresh plant samples (whole plant parts) collected were washed individually under running tap water and dried in an oven at 40^0c for 3 days. The plants were collected and chapped small pieces of shadow dry. The dried plant materials were ground into powder. About 10 g of dry powered plant material from each plant was extracted by soxhlate apparatus using

methanol solvent. The plant extracts was then concentrated using a rotary evaporator and the concentrated residual extracts were stored at 4^0c in a dry airtight container until further use.

2.3. Phytochemical Screening

Preliminary phytochemical analysis was carried out for methanol extracts of ***Heliotropium indicum*** as per standard methods described by Brain and Turner 1975 and Evans 1996.

2.3.1 Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used to test the presence of alkaloids.

a) Mayer's test

Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

Mayer's reagent

Mercuric chloride (1.358g) is dissolved in 60ml of water and potassium iodide (5g) is dissolved in 10ml of water. The two solutions are mixed and made up to 100ml with water.

b) Wagner's test

Filtrates were treated with wagner's reagent. Formation of brown/ reddish brown precipitate indicates the presence of alkaloids.

Wagner's reagent

Iodine (1.2g) and potassium iodide (2g) is dissolved in 5ml of water and made up to 100ml with distilled water.

2.3.2. Detection of Flavonoids

a) Lead acetate test

Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

b) H_2SO_4 test

Extracts were treated with few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

2.3.3. Detection of Steroids

Liebermann- Burchard test

2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The colour changed from violet to blue or green in some samples indicate the presence of steroids.

2.3.4. Detection of Terpenoids

Salkowski's test

0.2g of the extract of the whole sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inner face was indicates the presence of terpenoids.

2.3.5. Detection of Anthroquinones

Borntrager's test

About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones.

2.3.6. Detection of Phenols

a) Ferric chloride test

Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

b) Lead acetate test

Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

2.3. 7. Detection of Saponins

Froth test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

2.3.8. Detection of Tannins

Ferric chloride test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green colour formation indicates the presence of tannins.

2.3.9. Detection of Carbohydrates

Fehling's test

0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

Fehling's solution A: Copper sulphate (34.66g) is dissolved in distilled water and made up to 500ml using distilled water.

Fehling's solution B: Pottassium sodium tartarate (173g) and sodium hydroxide (50g) is dissolved in water and made up to 500ml.

2.3.10. Detection of Oils and Resins

Spot test

Test solution was applied on filter paper. It develops a transparent appearance on the filter paper. It indicates the presence of oils and resins.

2.4. Antibacterial Activity

2.4.1. Procedure

Screening of antibacterial activity

Totally four bacterial strains were used throughout investigation All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure.

2.4.2. Preparation of inoculums

Stock cultures were maintained at $4^{o}C$ on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at $37^{o}C$ and $25^{o}C$ respectively. The cultures were diluted with fresh Muller-Hinton broth to achieve optical densities corresponding to 2.0×10^{6} colony forming units (CFU/ml) for bacteria.

2.4.3. Antimicrobial susceptibility test

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antimicrobial activity. *Invitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media

into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37^{o}C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The standard disc is chloramphenicol.

2.5. In Vitro anticancer Activity (cytotoxicity activity)

2.5.1. MTT assay

MTT-Assay-Chemicals

3-(4,5–dimethyl thiazol–2–yl)–5–diphenyltetrazolium bromide (MTT), Fetal Bovine serum (FBS), Phosphate Buffered Saline (PBS), Dulbecco's Modified Eagle's Medium (DMEM) and Trypsin were obtained from Sigma Aldrich Co, St Louis, USA. EDTA, Glucose and antibiotics from Hi-Media Laboratories Ltd., Mumbai. Dimethyl Sulfoxide (DMSO) and Propanol from E.Merck Ltd., Mumbai, India.

2.5.1. Cell Lines and Culture Medium

MCF- 7 (Breast cancer cell line) cell cultures were procured from National Centre for Cell Sciences (NCCS), Pune, India. Stock cells were cultured in Dulbecco's modified Eagle's medium (DMEM). Medium was supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/ml), streptomycin (100 µg/ml) and amphotericin B (5 µg/ml) in an humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks and all experiments were carried out in 96 microtitre plates (Tarsons India Pvt. Ltd., Kolkata, India).

2.5.3. Preparation of Test Solutions

For cytotoxicity studies, each weighed test drugs were separately dissolved in distilled DMSO and volume was made up with DMEM supplemented with 2% inactivated FBS to obtain a stock solution of 1 mg/ml concentration and sterilized by filtration. Serially two fold dilutions were prepared from this for carrying out cytotoxic studies.

2.5.4. Determination of Cell Viability by MTT Assays

The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% FBS and were used for the determination of cell viability by MTT assays as described by Francis and Rita (1986) respectively. The absorbance was measured using a microplate reader at a wavelength of 540 nm. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values is generated from the dose-response curves for each cell line.

$$\% \text{ Growth inhibition} = 100 - \frac{\text{Mean OD of individual test group}}{\text{Mean OD of control group}} \times 100$$

3. RESULT AND DISCUSSION

The phytochemical screening of methanolic extracts revealed that the leaf and stem of *Heliotropium indicum* was rich in secondary metabolites such as Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Saponin and carbohydrates (Table. 1). This observation can be attributed to the presence of metabolites like Alkaloids, Saponins and Tannins whose antibacterial activity has been previously documented.[13] This plants leaves and stem showed antimicrobial activity against rich in *Escherichia coli* compare those four microorganisms (*Bacillus subtilis, Streptococcus mutants, Escherichia coli and Bacillus cereus).* The Maximum clear zone of inhibition was found in *Escherichia coli* bacteria (Table-2 and Figure-1). The cell lines in the medium expressed a significant result. Among the concentrations studied 1000µg/ml of extract shown a maximum inhibition of 84.75% and the least percentage of 44.83 was observed in 62.5µg/ml concentration of extract. (Tables: 3)**.** The inhibition action of extract may be due to the Phytochemical such as alkaloids, flavonoids and terpenoids. The inhibitory for 50% of cells (CTC_{50}) was found to be 97.54 µg/ml (Table- 3, Figure: 3). The CTC_{50} value was calculated by plotting the concentration of extract in X- axis and percentage of inhibition in Y- axis. From this graph the concentration from which 50% of inhibition of cell growth was calculated and kept as CTC_{50} value (Figure: 2). Aqueous extract from *Heliotropium indicum* leaves showed anticancer activity against Schwartz leukemia (ascites) at a dose of 200mg / kg.[10]

Table: 1 Qualitative phytochemical analysis

Phytochemicals	Observations	Extracts	
		H.I.S.M	H.I.L.M
Alkaloids	Cream colour	+	+

Mayer's test Wagner's test	Reddish brown solution/ precipitate		
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	-	+
Phenols Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	+	+
Terpenoids Salkowski test	Reddish brown precipitate	-	+
Saponin	Stable persistant	+	-
Steroids Liebermann-Burchard test	Violet to blue or Green colour formation	-	+
Tannin	Brownish green / Blue black	-	-
Carbohydrates	Yellow / brownish / blue / green colour	+	+
Arthroquinone Borntrager's test	Pink colour	-	-
Oils & Resins	Filter paper method	-	-

Tables: 2 Antibacterial Activity

S.no.	Name of Organism	Control (mg/ml)	H.I.L (mg/ml).	H.I.S. (mg/ml)	S.H.L. (mg/ml)	S.H.S. (mg/ml)
1.	*B.subtilis*	16	19	21	20	20
2.	*S.mutans*	16	20	18	20	19
3.	*E.coli*	18	27	25	22	25
4.	*B.cereus*	19	22	20	17	19

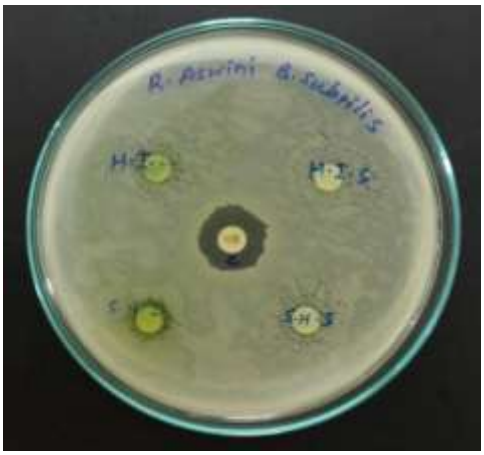


Plate 1: *B.subtilis*

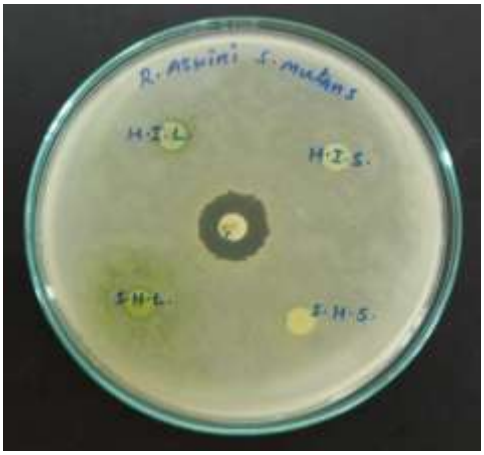


Plate 2: *S.mutans*ssss

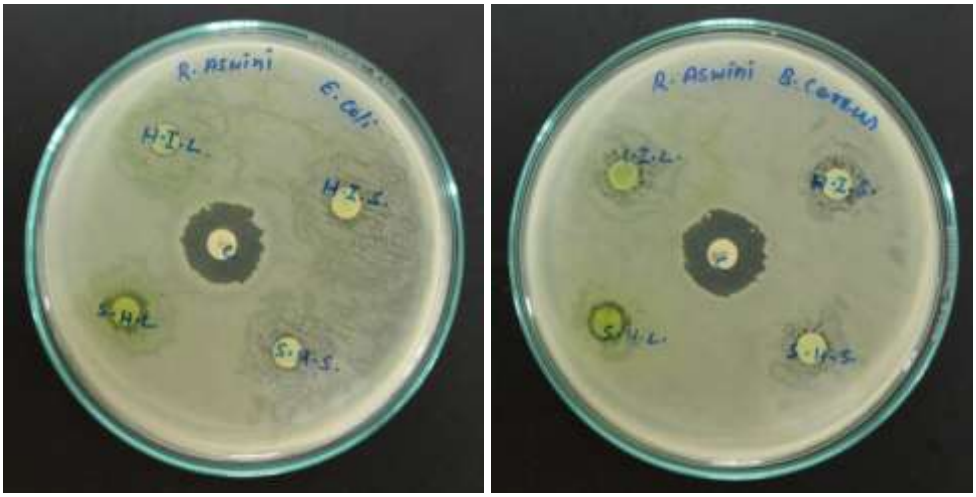


Plate 3: E.Coli **Plate 4: *B.cereus***

Figure 1: Antibacterial activity

Table: 3 MCF – 7 Cell Line (HIL)

S.No	Concentration	% CTC_{50}	CTC_{50}
1	1000	84.75	97.54
2	500	73.06	
3	250	64.91	
4	125	57.25	
5	62.5	44.83	

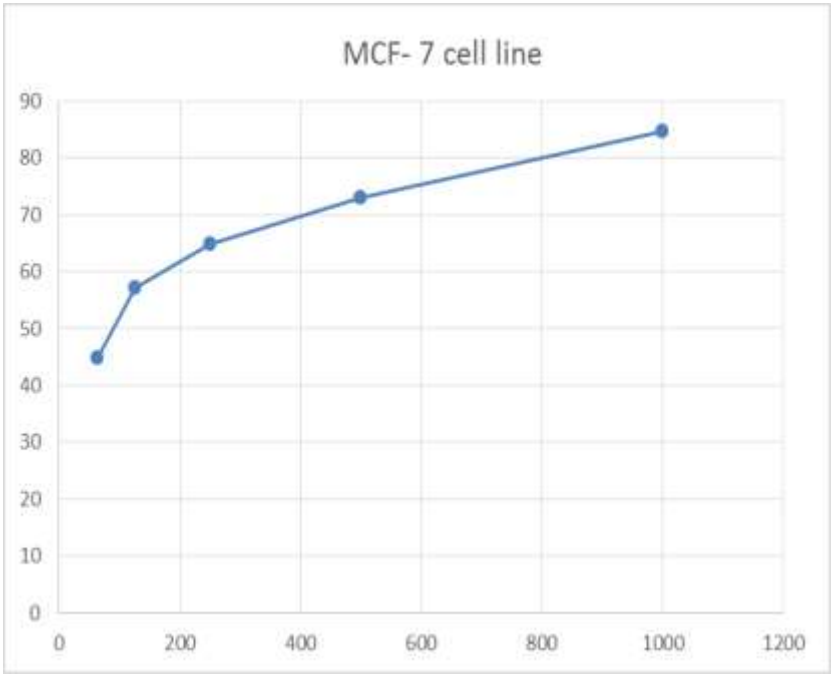
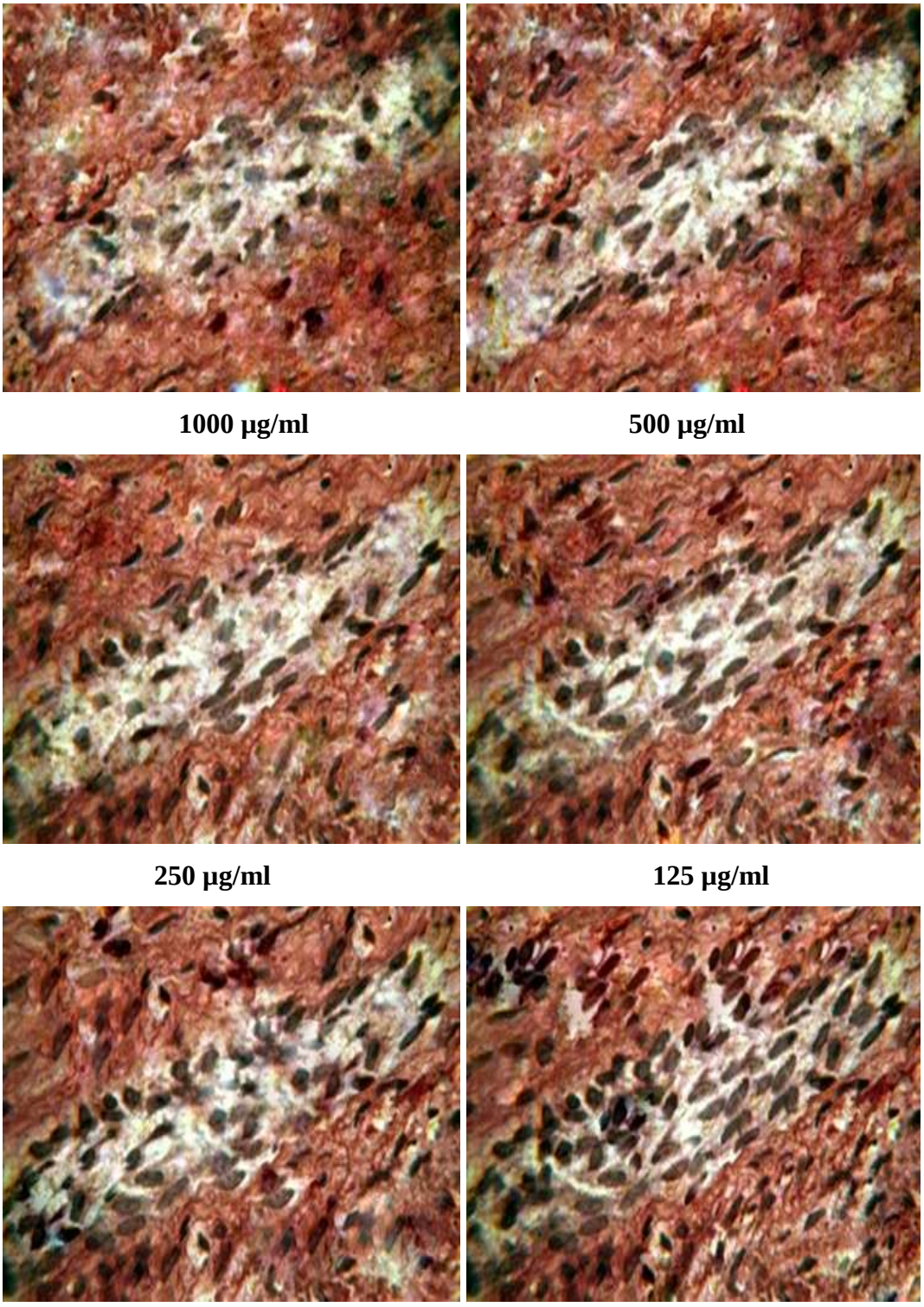


Figure: 2: CTC_{50} value of *Heliotropium indicum* MCF- 7 cell line



1000 µg/ml **500 µg/ml**

250 µg/ml **125 µg/ml**

60.5 µg/ml **31.25 µg/ml**

Figure: 3 In- vitro Cytotoxicity concentration of MCF – 7 Cell Line.

CONCLUSION

In the present study almost minimum number of secondary metabolite were observed in *heliotropium indium* such as Alkaloids, Flavonoids, Phenols, Terpenoids, Steroids, Saponin and carbohydrates. These plants have potential for antibacterial agent against *Bacillus*

subtilis, Streptococcus mutants, Escherichia coli and Bacillus cereus. The studies therefore consider that the 80% methanolic extract of *Heliotropium indicum* L leaf significant inhibitor of Breast cancer.

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