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Phytochemical and Biological Screening of Organic Solvent Extracts of *Ipomoea Pes-Caprae*flower

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Abstract

Phytochemicals are extensively used as medicinal compounds for treatment of various ailments all over the world. Ipomoea pes-caprae is a medicinal plant and utilizing naturally derived plant product or drug is less dangerous. The phytochemical analysis, antioxidant, antimicrobial and anticancer activity of Ipomoea pes- caprae flower extract were studied. Phytochemical analysis reported the presence of carbohydrate, protein, amino acids, flavonoids, steroids, tannins, saponins and glycosides. Carbohydrate, protein, amino acid, alkaloid and flavonoids are the major components in the ethanolic flower extract. Ferric reducing antioxidant power (FRAP) assay, Nitric Oxide scavenging assay, and Hydroxyl scavenging assay of the ethanolic extract showed good scavenging and reducing activity and it possessed good antioxidant efficacy. The flower extracts were tested against three gram positive bacteria Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, three gram negative bacteria Proteus vulgaris, Klebsiellapneumoniae, Escherichia coli and three fungus Aspergillus flavus, Aspergillus niger and Penicillium sp., by disc diffusion method. The ethanolic extract showed antimicrobial activity against all pathogens and maximum zone of inhibition of 21 mm was recorded with Staphylococcus aureus and 20 mm with Penicillium sp. As a result of potential antimicrobial activity and bioactive compounds present in the ethanolic flower extract, it was tested against lung cancer cell line - A549 by MTT assay and it showed effective cell inhibition with IC50 value 55.2527µg/mL. It was also tested for the cytotoxicity against normal cell line-L929 and observed with IC50 value 161.45. This study revealed that ethanolic extract of Ipomoea pes-caprae flower can be evolved as a new drug

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for treating cancer and microbial diseases.

Keywords : Antimicrobial, Antioxidant, Cytotoxicity, Ipomoea pes-caprae, Phytochemical.

INTRODUCTION

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cos et al., 2006). The medicinal properties of plants are attributed to the secondary metabolites synthesized in the plants and increasing attention has been directed towards the use of these for the treatment of many infectious diseases (Okunade 2002), antimicrobial, anticancerous, anti-inflammatory and for many other medicinal activities (Huang et al., 2008). *Ipomoea pes-caprae*(L.) R.Br. also known as Beach Morning Glory or Goat's Foot, is a common tropical creeping vine and belongs to the family Convolvulaceae and has around 600 species. *I. orizabensis*produced strong activity against sarcoma 37 (Belkin et al., 1952). *Ipomoea carnea*leaves possesses a strong anti-inflammatory activity (Khalid et al., 2011) and the plant possesses various bioactive compounds such as glycosides, alkaloids, reducing sugars, flavonoids, fatty acid, esters, alcohol and tannins (Satish et al., 2016). Antioxidant, analgesic, anti-inflammatory, antispasmodic, antihistaminic, immunostimulant, insulinogenic, hypoglycemic (Manigauhaet al., 2010) and antimicrobial properties (Bragadeeswaranet al., 2010) of*I. pes-caprae*have been documented. Flower of *Ipomoea murucoides*showed antibacterial activity against strains of *Staphylococcus aureus* possessing multidrug resistance (Cherigoet al., 2009). The fresh flower of *Ipomoea carnea*possesses wound healing activity (Ambigaet al., 2007). Nightingale Sheebaet al., (2019) reported that the aqueous flower extract of *Ipomoea pes-caprae*could prove as potential therapeutic agents that can target specific bacteria and cancer cells. Based on the previous report, the present study

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was designed to extract and screen various bioactive compounds present in the different solvent extracts of *Ipomoea pes-caprae*flower and to analyze its pharmacological properties.

MATERIALS AND METHODS

Collection and extraction of plant material

The flowers were collected from Anjugram sea shore, Kanyakumari District in the early morning hours from September to November, 2019.The plant was authenticated by Dr. P. Nagerndra Prasad, Head, Department of Biotechnology, Sri Paramakalyani College, Alwarkurichi, Tirunelveli. The flowers were cut into small pieces and to 5 g of flower, 10 ml of solvent like methanol, ethanol, petroleum ether, n- Butanol and chloroform were added separately and grounded with motor and pestle. The extracts were boiled at 60°C for 3 hrs, kept overnight at 37°C and then filtered with Whatman No. 1 filter paper. The extracts were dried and stored at -20°C. Extractions were done by the modified method of Senthilet al., (2016).

Qualitative phytochemical screening of *Ipomoea pes-caprae*flower extract

Phytochemical screening of different extracts of the flower was subjected to qualitative phytochemical test for the presence of various classes of active chemical constituents such as carbohydrate, protein, amino acid, steroids, saponins, tannin, glycosides, alkaloid, flavonoid and phenol using standard procedures of Mukherjee (2008).

Antioxidant activity of solvent extracts

Hydroxyl radical scavenging activity

The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L-1, pH 7.4), 0.2 mL of a sample of different concentrations (2, 4, 8, 12, 16, 20 mg mL-1), 0.2 mL of

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EDTA (1.04 mmol L-1), 0.2 mL of $FeCl_3$(1 mmol L-1), and 0.2 mL of 2-deoxyribose (60 mmol L-1). The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 mL of ascorbic acid (2 mmol L-1) and 0.2 mL of H_2O_2 (10 mmol L-1). After incubation at 37 °C for 1 h, 2 mL of cold thiobarbituric acid (10 g L-1) was added to the reaction mixture followed by 2 mL of HCl (25%). The mixture was heated at 100 °C for 15 min and then cooled down with water. The absorbance of solution was measured at 532 nm with a spectrophotometer (Deep vision, 2373, Chennai, Tamilnadu, India). The hydroxyl radical scavenging capacity was evaluated with the inhibition percentage of 2-deoxyribose oxidation on hydroxyl radicals (Halliwell et al.,1987).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of solvent extracts of samples was estimated as described (Pulido *et al.*, 2000), FRAP reagent (900µL), prepared freshly and incubated at 37°C, was mixed with 90µL of distilled water and 30µL of test sample or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37°C for 30 minutes in a water bath. The final dilution of the test sample in the reaction mixture was 1/34. The FRAP reagent contained 2.5mL of 20 µmol/L 2, 4, 6-tripyridyl-2-triazine (TPTZ) solution in 40µmol/L HCl plus 2.5mL of 20 µmol/L $FeCl_3 \cdot 6H_2O$ and 25mL of 0.3 mol/L acetate buffer (pH 3.6) as described by Siddhuraju and Becker (2003) at the end of incubation, the absorbance readings were taken immediately at 593nm, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2,000µmol/L, ($FeSO_4 \cdot 7H_2O$) were used for the preparation of the calibration curve. The FRAP value is expressed as mmol Fe (II) equivalent/mg extract.

Nitric oxide generation and assay of Nitric oxide scavenging method:

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SNP Sodium nitroprusside (10mM) in phosphate buffer saline (PBS) was mixed with different concentration of extract (25-100µg/ml) dissolved in methanol, ethanol, petroleum ether, n- Butanol and chloroform and incubated at 25°C for 180 minutes. The samples from the above were reacted with Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm and referred to the absorbance of ascorbic acid, used as a positive control treated in the same way with Griess reagent (Green et al.,1982).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of the sample was performed using a Shimadzu GCMS-QP2010 gas chromatograph mass spectrometer interfaced with a Turbo Mass quadrupole mass spectrometer, fitted with an Rtx-5 fused silica capillary column (30 X0.25 mm, with 1cm film thickness) (Tamil nadu, India). The oven temperature was programmed from 100°C to 320°C at 100ºC/min and a hold for 10 min. Helium was used as carrier gas at flow 1.0 mL/min. The injector temperature was 250ºC, injection size 1 µL neat, with split ratio 1:10. The interface and MS ion source were maintained at 320ºC and 200ºC respectively and the mass spectra were taken at 70eV with a mass scan range of 40-700 amu (atomic mass unit). Data handling was done using GCMS solution software (Senthil et al., 2016).

Collection of test micro organisms

Test microorganisms *Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Proteus vulgaris, Klebsiellapneumoniae, Escherichia coli, Aspergillus flavus, Aspergillus*

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*niger*and *Penicillium*sp. were obtained from Inbiotics, Research Centre, Nagercoil, Tamilnadu, India.

Antimicrobial Assay

Antimicrobial Assay was determined against bacteria and fungi following the method of Bauer et al., (1966). The medium was prepared by dissolving 33.9 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121°C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured onto 100 mm petriplates (25 ml/plate) swabbed with pathogenic bacteria culture. The sample loaded disc was then placed on the surface of Muller-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters. Streptomycin disc were used as positive control and sterile discs were used for negative control.

In vitro anticancer study-cell culture and MTT Assay

Cells was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigmaaldrich, USA). Cultured cell lines were kept at 37ºC in a humidified 5% CO2 incubator (NBS Eppendorf, Germany). The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.After 24 hours the growth medium was removed, freshly prepared each compounds in 5% DMEM were (100µL, 50µL, 25µL, 12.5µL, 6.25µL in 500µl of 5% DMEM) added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO2 incubator. Non treated control cells were also maintained.Entire

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plate was observed after 24 hours of treatment in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity. Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization..After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37ºC in a humidified 5% CO2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide,DMSO, Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

RESULTS AND DISCUSSION

Plants are an important source of potentially useful bioactive compounds that can be tapped for the development of new chemotherapeutic agents (Mahesh & Satish, 2008). *Ipomoea pes- capra*is a medicinal plant that has been reported to be a vital source of antioxidant phytochemical (Devall, 1992). Flowers form an integral part of the plant and are known to contain a wide variety of phytochemicals. In the present study, the different solvent extracts of *Ipomoea pes-capra*eflower were analyzed for its bioactive compounds and the antioxidant, antibacterial and anti- proliferative activities.

The phytochemical analysis of the flower reported the presence of carbohydrate,

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protein, amino acids, flavonoids, steroids, tannins, saponins and glycosides. Carbohydrate, protein, amino acid, alkaloid and flavonoids are the major components in the ethanolic flower extract (Table: 1). Similar findings were reported in *Ipomoea sepiaria*where a good quantity of phenol and flavonoid content were noted in the methanolic and ethanolic extracts which showed higher flavonoid content with greater antioxidant and antibacterial activity (Naz et al., 2017). Presence of phytochemicals like alkaloids, flavonoids, terpenoids, glycoside, reducing sugars, saponins, steroids and tannins have been reported in the solvent extract of *Ipomoea indica*(Yuvarani&Selvam, 2018).

The antioxidant property of *Ipomoea pes-caprae*flower extract was determined using ferric reducing activity, nitric oxide scavenging and hydroxyl radical scavenging assays. Free radicals play an important role in biological tissues and most of the diseases in human body are initiated by the production of free radicals. The risk of free radicals can be reduced by the intake of antioxidant food supplements and it has been documented that the phenolic and flavonoid compounds present in most plant extracts possess antioxidant properties (Ferreira et al., 2007). Reducing power activity is the measure of strength to neutralize the free radical. The medicinal value of the plant product is assessed on the basis of reducing power potential (Naz et al.,

2017). Figure 1(a) shows the percentage of reducing power of different flower extract of *Ipomoea pes-caprae*. The reducing power assay evaluates the electron donation ability of an antioxidant. Maximum percentage of reducing activity was noted with ethanolic extract of *Ipomoea pes-caprae*flower followed by the methanolic extract. The high absorbance intensity can be correlated with the reducing ability of either the phenol or flavonoids present in flower. High free radical scavenging activity was observed in all the extracts tested and maximum

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activity was noted at 100

µg concentration (Figure 1(b) and (c). These results indicate that the percentage of scavenging activity is proportional to the concentration and is in agreement with the previous report (Lee et al., 2016). The high antioxidant activity of flowers may be attributed to the level of flavonoid (Chantarudee et al., 2012). *I. purpurea*the flowers of this species showed antioxidant activity (Kano et al., 2005). Protective effect of plants may be due to the presence of natural antioxidant compounds (Choi et al., 2007). The reducing capacity of a compound serve as a significant indicator of its potential antioxidant activity (Hsu et al., 2006) and it can effectively reduce the oxidative stress (Nantitanon et al., 2010).

As a result of the high bioactive potential noted in the ethanolic extract of *Ipomoea pes- caprae*flower, the GC-MS analysis was carried out to investigate the presence of potential bioactive compounds and its biological activities were reviewed and demonstrated (Table 2). The GC-MS analysis revealed the presence of 19 compounds and among these 6 compounds (Methyl salicylate, N,N,N′,N′-Tetramethyl-1,3-propanediamine, Diethyl Phthalate, n-Hexadecanoic acid, 9-Octadecenoic acid, (E) and cis-9-Hexadecenal) have been reported to possess biological activities like antioxidant, antimicrobial and anticancer property (Figure 2). This study was supported by the previous GC-MS report of *Ipomoea sepiaria*leaves with 20 compounds and many of these compounds have antimicrobial, antidiabetic, anti-inflammatory, antioxidant and anticancerous activities (Senthil et al., 2016).

Antimicrobial activity of *Ipomoea pes-caprae*flower extracts were studied against nine pathogenic microbes (*S. aureus, B. subtilis, S. mutans, P. vulgaris, K. pneumonia, E. coli, A. flavus,*

*A. niger and Penicillium*sp.). Antimicrobial activity of solvent extract was assessed in terms of

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zone of inhibition of microbial growth). Highest inhibition zone was observed in ethanolic extracts (21 mm) against *Staphylococcus aureus* bacteria and chloroform extract showed 21 mm against *Penicillium*species. The results showed the potential of microbial growth inhibition by ethanolic extract against all the tested microbes (Figure: 3(a, b) and Table: 3,4). Aqueous extract of *Ipomoea pes-caprae*flower exhibited antimicrobial activity against both Gram positive and negative bacteria moderately and high cytotoxic effect against A549 cell line as evident from the data already published (Sheeba et al., 2019). In the present study the ethanolic extract showed a cytotoxic effect similar to aqueous extract whereas the antimicrobial activity of ethanolic extract was comparatively higher. The antibacterial activity of this medicinal plant may be due to the alkaloids and flavonoids (Ríos &Recio, 2005) or methyl salicylate (Oloyede 2016), diethyl phthalate (Premjanu&Jayanthy, 2014) and cis-9-Hexadecenal (Mujeeb et al., 2014). Rashid et al., (2002) reported that the crude Petroleum ether, chloroform and ethyl acetate extracts of *Ipomoea turpethum*showed antimicrobial activity against gram positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Sarcinalutea, Streptococcus hemolyticus, Pseudomonas aeruginosa, Sarcinasarcinaceae*) and gram negative bacteria (*E. coli, Shigelladysenteriae, Shigellashiga, Shigellaboydii, Shigellasonnei, Shigellaflexneriae, Salmonella typhi*).

Based on this study and previous review, it is clearly observed that ethanolic extract contains more amount of flavonoids with antioxidant property and *Ipomoea* plants showed good anticancer activity. The anti-proliferative effect of ethanolic extract of *Ipomoea* was tested against lung cancer line A549 by MTT assay and it also showed effective cell inhibition with IC_{50} value 55.2527µg/ml. Further it was analyzed with L929 normal fibroblasts cell line to find out the cytotoxicity of the ethanolic extract and it showed low toxicity with IC_{50} value

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161.45µg/ml (Figure: 4,5(a, b) and Table: 5).

*I. orizabensis*produced strong activity against sarcoma 37 (Belkin et al., 1952) and $CHCl_3$ and MeOH extracts of *I. purga*had a significant inhibitory effect (ED50 <4 µg/mL) against the human nasopharyngeal carcinoma and breast cancer cell cultures (Pereda-Miranda et al., 2003). Methanolic extract of *Ipomoea pes-caprae*exhibited a stronger antitumor effect against melanoma tumor as compared to aqueous and petroleum ether extracts (Manigauha et al., 2015). The present study has documented the presence of potential bioactive compounds in *Ipomoea pes-caprae*flower that can be targeted againstpathogenic microbes and cancer cells. The particular constituent responsible for antimicrobial and anticancer activity can be isolated and docking studies can be carried out to understand the structure function relationship which in turn will help to isolate the biomolecule that can be used for clinical trials.

CONCLUSION

The present study revealed that the ethanolic extract of *Ipomoea pes-caprae*flower has bioactive compounds that can be synthesized as a new drug for treating microbial diseases and cancer cells.

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*indica*Plant Leaf Extracts. *International Journal of Current Pharmaceutical Research*, 2018; 10(2): 86-88.

Table – 1: Phytochemical analysis of various solvents extracts of flower *Ipomoea pes-caprae*

Phytochemical Compounds	Methanol	Ethanol	Petroleum ether	n- Butanol	Chloroform
Carbohydrate	++	+++	-	-	-
Protein	++	+++	-	+	-
Amino acid	++	+++	-	+	-
Steroids	-	-	-	-	-
Saponins	-	++	-	-	-
Tannin	-	+	-	-	-
Glycosides	-	+	-	-	-
Alkaloid	+++	++	++	-	++
Flavonoids	+	++	++	+++	++
Phenols	+	+	+	+	+++

+ □ Low, ++ □ Moderate, +++ □ High, - □ Absent

Table 2: GC-MS analysis of ethanolic extract of*Ipomoea pes-caprae* flower and its biological activities

Compound Name	Biological activity
Methyl salicylate	Antimicrobial and Antioxidant (Oloyede, 2016)
N,N,N′,N′-Tetramethyl-1,3-propanediamine	Antimicrobial (Obłąk, 2014)
Diethyl Phthalate	Antimicrobial (Premjanu, 2014)
n-Hexadecanoic acid	Antioxidant, Pesticide, Antifibrinolytic (Ponnamma&Manjunath, 2012)
9-Octadecenoic acid, (E)	Cancer preventive, Antiinflammatory (Rajalakshmi& Mohan, 2016)
cis-1,4-Dimethyl-3-piperidinol	No activity found
6-(Benzyloxy)-1H-indole-2-carboxylic acid	No activity found
cis-9-Hexadecenal	Antimicrobial (Mujeeb, 2014)

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Table 3: Antibacterial activity of various extracts of *Ipomoea pes-caprae* flower

Bacteria Strains	Zone of inhibition (mm)						
	Methanol	Ethanol	Petroleum Ether	n-Butanol	Chloroform	Positive	Negative
S. aureus	16	21	-	12	11	25	-
B. subtilis	-	20	-	16	10	13	-
S. mutans	7	19	-	13	13	27	-
P. vulgaris	-	16	-	16	11	17	-
K. Pneumoniae	7	11	-	-	-	13	-
E. coli	-	7	10	-	13	24	-

Table 4: Antifungal activity of various extracts of *Ipomoea pes-caprae* flower

Fungi Strains	Zone of inhibition (mm)						
	Methanol	Ethanol	Petroleum Ether	n-Butanol	Chloroform	Positive	Negative
Aspergillus flavus	10	10	-	14	-	20	-
Aspergillus niger	14	15	-	12	-	16	-
***Penicillium* sp.**	13	20	19	10	21	19	-

Table 5: Percentage of cell viability and IC_{50} values of two cell lines treated with ethanolic extract of *Ipomoea pes-caprae*flower

Cell lines	Cell Viability (%)					IC_{50} value µg/ml
	6.25	12.5	25	50	100	
A549	80.49	76.61	63.24	52.75	24.88	55.25
L929	94.32	90.43	89.88	73.17	69.64	161.45

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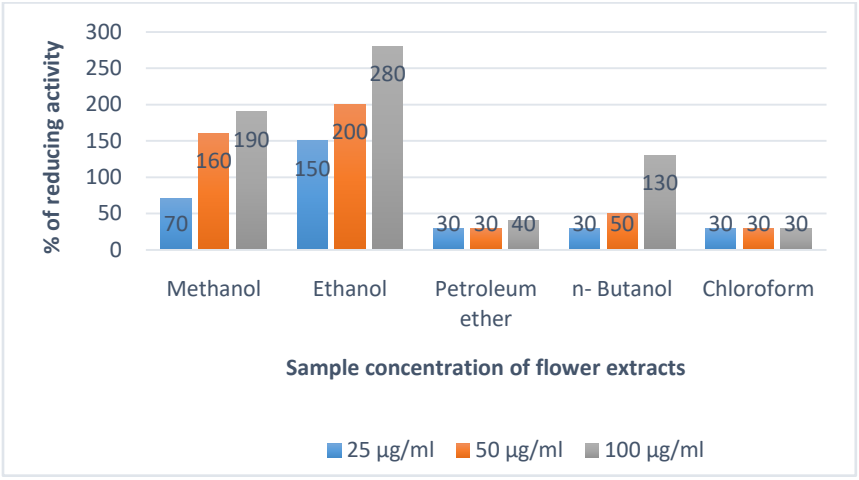

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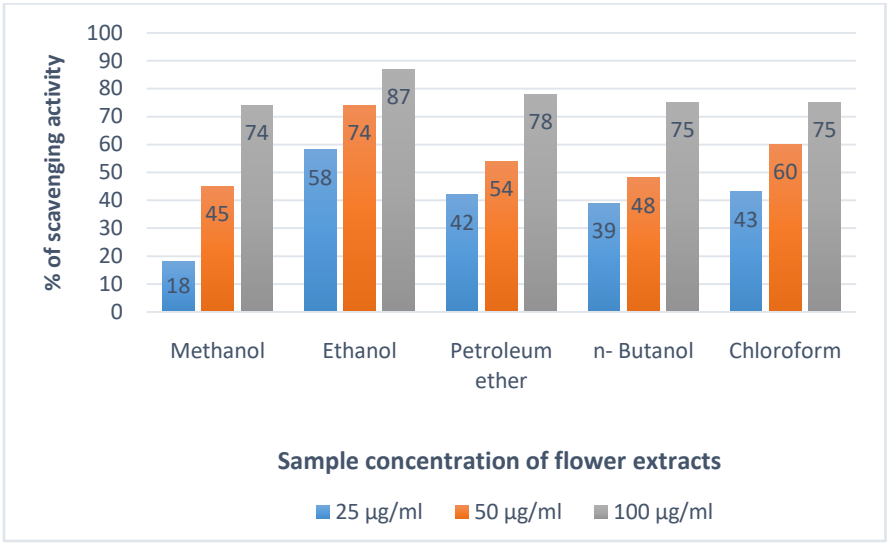

(b)

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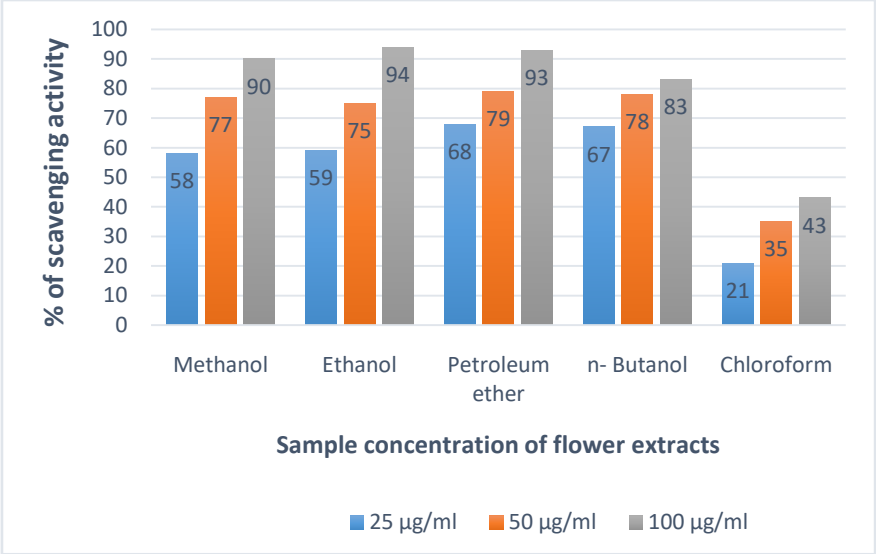

(c)

Figure 1: a) Ferric reducing antioxidant power (FRAP) activity, b) Nitric Oxide scavenging assay and c) Hydroxyl radical scavenging activity of *Ipomoea pes-caprae* flower extracts

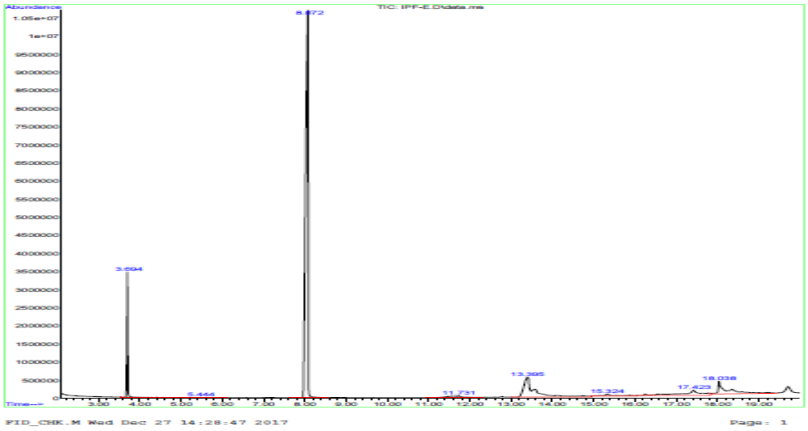

Figure 2: GC-MS analysis of ethanolic extract of*Ipomoea pes-caprae* flower

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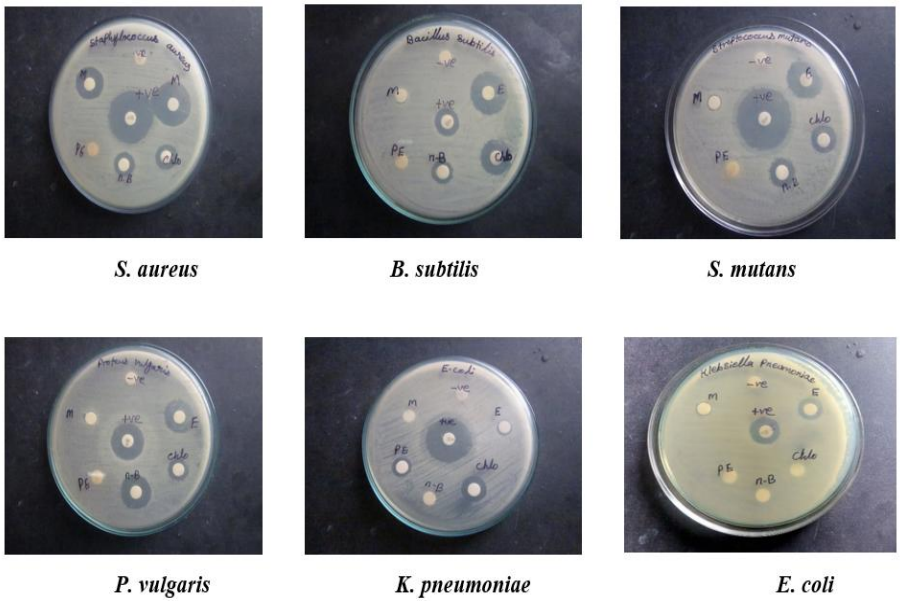

S. aureus *B. subtilis* *S. mutans*

P. vulgaris *K. pneumoniae* *E. coli*

M = Methanol, E = Ethanol, PE = Petroleum Ether, n-B = n-Butanol, C = Chloroform

(a)

Penicillium **sp.** *Aspergillus flavus* *Aspergillus niger*

M = Methanol, E = Ethanol, PE = Petroleum Ether, n-B = n-Butanol, C = Chloroform

(b)

Figure 3: a) Antibacterial and b) Antifungal activity of different solvent extracts of *Ipomoea pes-caprae* flower

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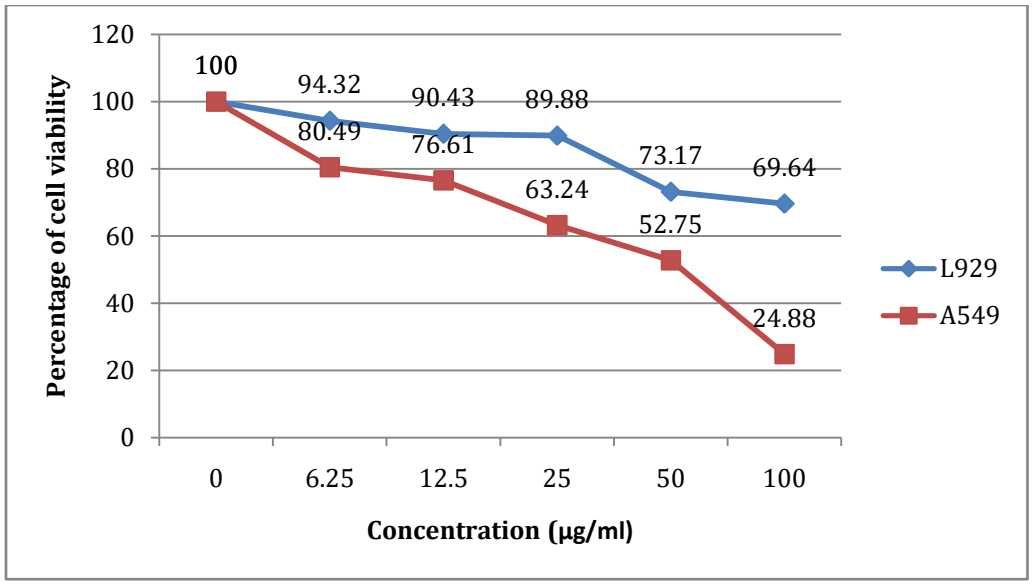
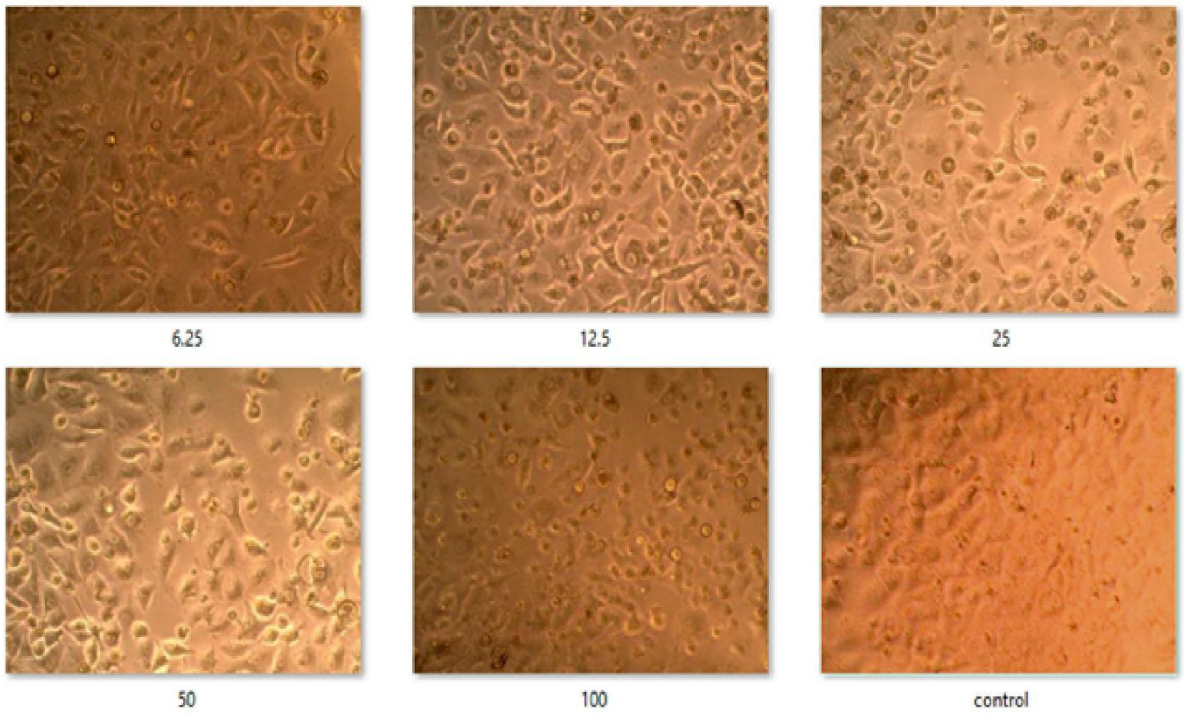


Figure 4: Percentage of cell viability and IC_{50} values of two cell lines treated with ethanolic extract of *Ipomoea pes-caprae*flower



(a)

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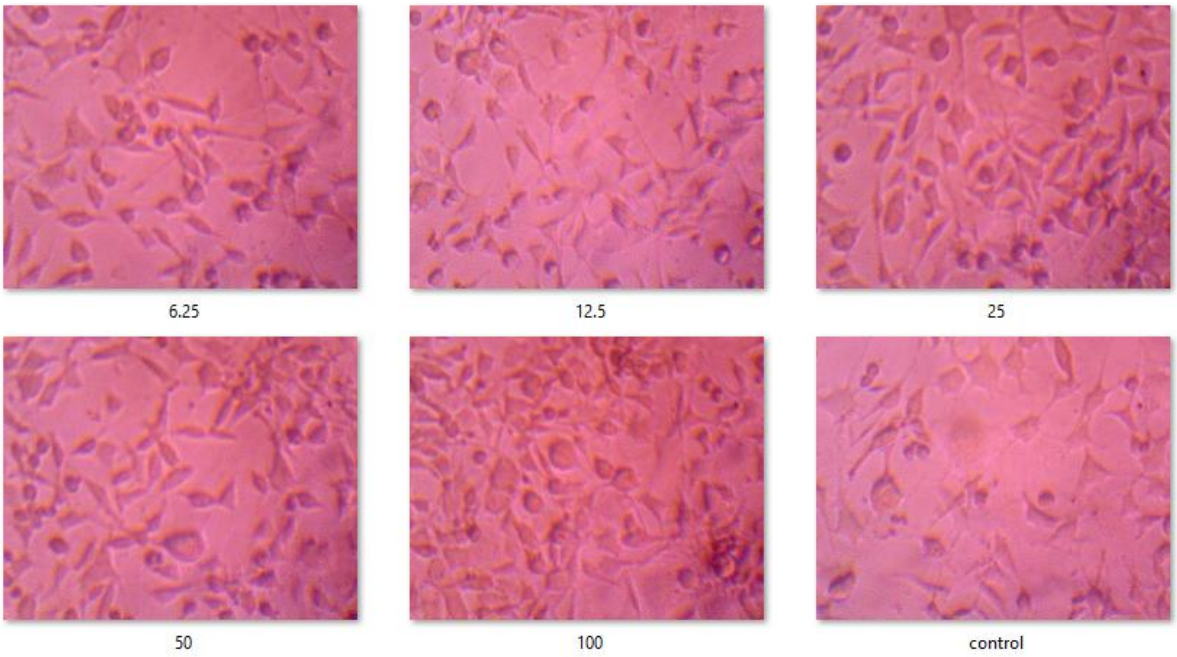

(b)

Figure 5: a) Microscopic image of A549 cell lines and b) L929 cell line treated with ethanolic extract of flower in different concentrations (µg/ml)