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Phytochemical Analysis and Biomedical Applications of *Ipomoea pescaprae* Flower Extract-An *In Vitro* Study

Nightingale Sheeba, S[1], Vinoliya Josephine Mary, J[2*], Mary Mettilda Bai, S[3] and Jilian V. Paul[4]

[1]Research Scholar, [2,3]Assistant professors, [4]Teaching Assistant.

[1,2,3] Department of Zoology, Holy Cross College (Autonomous), Nagercoil.

[4]Department of Biomedical Sciences, Alagappa University, Karaikudi.

[1]Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli – 627 012, Tamil Nadu, India.

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Corresponding Author Email: vinoliya75@gmail.com

Abstract

Traditional remedies have a long-standing history in India and continue to provide very useful and applicable tools for treating various ailments. Ipomoea pes-caprae /Ipomoea biloba commonly known as morning glory which is found along the sea shore belongs to the family Convolvulaceae. Phytochemical analysis revealed the presence of protein, saponin, tannin, glycosides, alkaloid and flavonoid as the major components in the flower extract of Ipomoea pes-caprae. Aqueous extract of the fresh flower was evaluated for its antibacterial potential against Gram +ve and Gram –ve bacteria such as Staphylococcus aureus, Bacillus subtilis, Streptococcus mutans, Lactobacillus sp., Proteus vulgaris, Klebsiella pneumoniae, Escherichia coli and Proteus mirabilis. The aqueous extract inhibited most of the selected bacteria except Klebsiella pneumoniae. The anticancer activities of the flower of Ipomoea pes-caprae were evaluated against cell lines A549, HT29 and MCF7. High cytotoxic effect was observed against A549 cell line. Our results suggest that flower extracts are promising therapeutic agents that can target specific bacteria and cancer cells.

Keywords

Antibacterial, Antiproliferative, Cytotoxic, Phytochemical.

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INTRODUCTION

Medicinal plants are currently in considerable significance due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs [1].

Many of the plants used today were well known to the people of ancient cultures throughout the world and they were valued for their preservation and medicinal powers [2, 3]. The therapeutic efficacies of plants, for various diseases have been described by traditional herbal medicine practitioners [4]. Numerous natural drugs were reported from plants that were known to possess antimicrobial activity. *Ipomea pes-caprae* is a valuable medicinal plant which belongs to Convolvulaceae family [5]. This plant was found to be very important as a source for bioactive principle and natural antioxidants [6]. The medicinal value of Ipomoea plant is reported for their antimicrobial, anticancer, anti-inflammatory and other ailments [7]. The lyophilized leaf powder of sweet potato (*Ipomoea batatas*) strongly suppressed the growth of Gram positive and Gram negative bacteria [8].

The crude methanol extract of *Woodfordia fruticosa Kurz.* Flower was more active against Gram negative bacteria when compared to Gram-positive bacteria [9]. Constituents like tannins with significant antibacterial property enabled the extract to overcome the barrier in Gram negative cell wall [10]. The plant *I. pes-caprae* is reported for its analgesic, antioxidant and anti-inflammatory, antinociceptive activities, antispasmodic, immunostimulant, antihistaminic, hypoglycaemic, insulinogenic, [11] antifungal and antibacterial characteristics [12]. Also, this plant extract inhibits platelet aggregation, and cures diarrhoea, piles and vomiting [13] and is used in the treatment of boils, skin diseases, swelling, ulcer, wounds, carbuncle, menorrhagia, dropsy, hemorrhoids, flatulence, colic, dyspepsia, burning sensation and cramp.

Effects of plant extracts on cancer cell lines are well documented. *Moringa oleifera* extract exhibited cytotoxic effect on HeLa cells which was effective in dose dependent manner [14]. Flower extracts of *Ixora cocciea* are active against all cell lines including U937, Colo205, B16F10, HepG2 and HeLa at below 200 µg/ml concentration [15]. Various approaches are being tried through modulation of antitumor immune response, antitumor proteins and apoptosis for the treatment of cancer [16]. *Hibiscus sabdariffa L* flowers have high anticancer potential against HepG2 cell line. [17] *I. pes-caprae* was identified as a highly selective growth inhibitor of human melanoma and non-melanoma [18] neuroectodermal and malignant tumor cells and was reported to induce apoptosis in numerous cancer cell lines in vivo and in vitro [19]. The objective of this investigation is to evaluate the biomedical potential of aqueous extract of *Ipomoea pes-caprae* flower against few pathogenic bacteria and antitumor effect on A549, HT29 and MCF7 cancer cells.

MATERIALS AND METHODS

Collection and extraction of plant material

The fresh flowers of *Ipomoea pes-caprae* were collected from the coastal area of Kanyakumari District, Tamilnadu, India. They were immediately brought to laboratory and washed thoroughly with sterile double distilled water to remove the debris. The clean flowers were shade dried for three days and about 10 g of powdered samples were extracted with 300 ml of distilled water by using soxhlet apparatus and the extraction was carried out for 24 Hours at 60°C. The extract was filtered with Whatman No.1 filter paper, dried and stored at room temperature for bioassays [20].

Phytochemical analysis

Qualitative screening

Aqueous extract of flower was subjected to qualitative phytochemical test for the presence of various classes of active chemical constitutes such as carbohydrate, protein, amino acid, steroids, saponin, tannin, glycosides, alkaloid, flavonoid and phenol using standard procedures [21].

Test Micro organism

The test organisms used in this investigation include: *Staphylococcus aureus*, *Bacillus subtilis*, *Streptococcus mutans*, *Lactobacillus* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Proteus vulgaris* and *Proteus mirabilis* gifted by Inbiotics, Nagercoil. The bacterial isolates were cultured in a nutrient broth (Himedia, Mumbai, India) medium and incubated at 37 °C for 18 h.

Positive and negative control

Streptomycin (25 mg, Hi-media) was used as positive control for the bacterial strains and millipore water was used as negative control.

Antibacterial activity

Antibacterial activity of *Ipomea pes-caprae* was determined by disc diffusion method on Muller Hinton agar (MHA) medium [22-24]. The Muller Hinton agar plate was prepared and the inoculums were spread on solid plates with sterile swab moistened with the bacterial suspension. 25 µl of 25 µg, 50 µg, 75 µg and 100 µg of flower extract, 25 µl of double distilled water as negative control and streptomycin as positive control was used.

Anticancer activity

HT29, MCF7 and A549 cancer cell lines were obtained from National Centre for Cell Sciences (NCCS), Pune, India and maintained in Dulbecos modified Eagles medium (Gibco, Invitrogen). After 24 hours, the growth medium was removed completely. Then

freshly prepared flower extracts were suspended in 5% DMEM, diluted appropriately (100 µg, 50 µg, 25 µg, 12.5 µg, 6.25 µg in 1ml) and each concentration was loaded in triplicates to the respective wells and incubated at 37 ºC in a humidified 5% CO_2 incubator.

Cytotoxicity Assay by Direct Microscopic observation

Entire plate was observed at an interval of 24 h and continued up to 72 h in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and results were recorded. Any 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.

Cytotoxicity Assay by MTT Method

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 h of incubation period, the sample content in wells was removed and 30 µl of reconstituted MTT solution was added to the test and control wells. Then the plate was shaken well and incubated at 37ºC in a humidified 5% CO_2 incubator for 4 h. After 4 h, the supernatant was removed and 100 µl of DMSO was added and the wells were mixed gently. The absorbance values were measured by using micro plate reader at a wavelength of 570 nm (25).

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples X 100}}{\text{Mean OD of control group}}$$

RESULTS

Qualitative analysis of phytochemicals

Phytochemical analysis of aqueous extract of *Ipomea pes*-caprae flower is presented in Table 1. High concentration of alkaloids and flavonoids, moderate amount of tannins and proteins and trace amount of phenol, carbohydrate and glycosides were observed.

Antibacterial activity

The antibacterial activity of aqueous extract of flower *Ipomea pes-caprae* was investigated against four Gram + ve (*S. aureus, B. subtilis, S. mutans* and *Lactobacillus* sp.) and four Gram -ve (*K. pneumonia, E. coli, P. vulgaris* and *P. mirabilis*) bacterial strains and the results are presented (Table-2; Figure 1 and 2). *Ipomea pes-caprae* flower extract failed to inhibit the growth of the bacteria at 25µg and 50 µg/ml concentration. The tested bacteria were sensitive to the flower extract at 75 µg/ml concentration and maximum zone was recorded at 100 µg/ml concentration except *K. pneumonia.* Maximum inhibitory activity was observed with *B. subtilis* (12 mm) and moderate activity was noted with *S. aureus, S. mutans and Lactobacillus* sp.

Anticancer activity

The anticancer effect of aqueous extract of *Ipomea pes-caprae* was analysed against HT29, MCF7 and A549 cell lines and the results are shown in Table 3 and Figure 3, 4, 5, 6. The extract showed a concentration dependent effect on viability of all tested cell lines. The IC50 values obtained were 83.15 µg/ml (HT29), 75.15 µg/ml (MCF7) and 56.64 µg/ml (A549). The aqueous extracts of *Ipomea pes-caprae* flower showed potent activity against A549 cell line when compared to other cell lines.

DISCUSSION

Flowers are one of the important parts of the plants and are known to contain a wide variety of phytochemicals. In the present study, the flower extracts showed the presence of flavonoids, alkaloids, protein, amino acids, glycosides, tannin and phenol. Majority of the plant secondary metabolites have possessed remarkable health promoter effects such as antioxidants, antimicrobial and anticancer [26]. Drug discovery from plant parts still provides important new drug leads and many of which have potential clinical uses [27]. Phytomedicine has no apparent side effects that are associated with modern drug [28]. Evaluation of Ipomoea *pes-caprae* leaf and stem for pharmacognostic standards showed the presence of high amount of phyto constituents like alkaloids, phenols, steroids, tannins, flavonoids in leaf and stem [29]. The flowers of the *Ipomoea carnea* also show the presence of flavonoids, tannins, glycosides, alkaloids, carbohydrates and phenolic compound [30]. Analysis of the aqueous extract of *I. indica* leaves possesses bioactive compounds with antibacterial activity against the bacterial strains [31].

The flower extract of *Ipomoea pes-caprae* showed significant activity against various Gram +ve and Gram –ve bacterial pathogens. Antibacterial activity of flower extracts might be due to the presence of chemical constitutions in the extracts [32]. Antimicrobial property of flavanoids[33] and triterpenoids[34] has been observed in *Catharanthus roseus*[35]. Flavonoids have many pharmacological properties especially antimicrobial [36], anti-inflammatory [37], antioxidant [38, 39] and anti-tumour effects [40]. Tannins possess astringent, anti inflammatory, anti diarrhoea, antioxidant and antimicrobial activities [41]. Biological properties of

alkaloids are their toxicity against cells of foreign organisms. Alkaloids have been widely studied for their potential use in the elimination and reduction of human cancer cell lines [42]. Anticancer effect of aqueous extract of *Ipoma pes- caprae* was analysed against HT29, MCF7 and A549 cell lines and the extract was highly active against A549 cell line with IC50 value 56.64µg/ml and it also had effect on MCF7 and HT29. Flavonoids have antioxidant activity, protect cells against oxidative damage and reduce the risk of developing certain types of cancers [43]. Anticancer effect may be due to the alkaloids and flavanoids.

From the results it is confirmed that the aqueous extract of *Ipomoea pes-caprae* flower has antimicrobial property against Gram +ve and Gram –ve bacteria and also have anticancer effects against different cancer cells like A549, MCF7 and HT29.

Table 1: Phytochemical analysis of aqueous extracts of flower *Ipomea pes-caprae*

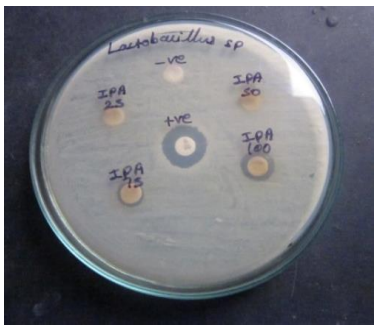
Phytochemicals	Concentration of constituents
Carbohydrate	+
Protein	++
Aminoacid	++
Steroids	-
Saponins	-
Tannin	++
Glycosides	+
Alkaloid	+++
Flavanoids	+++
Phenols	+

+: Low, ++: Moderate, +++: High, -: Absent

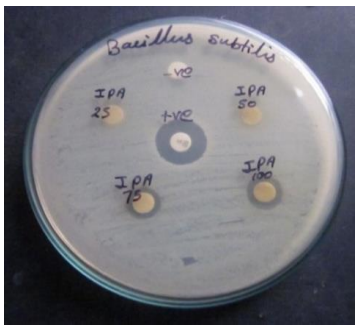
Table 2: Antibacterial activity of aqueous extracts of flower *Ipomea pes-caprae*

Strains	Zone of inhibition (mm)					
	IPA 25µg/ml	IPA 50 µg/ml	IPA 75 µg/ml	IPA 100 µg/ml	Positive	Negative
S. aureus	-	-	-	11	27	-
B. subtilis	-	-	11	12	15	-
S. mutans	-	-	8	10	15	-
Lactobacillus sp.	-	-	8	11	13	-
K. pneumonia	-	-	-	-	24	-
E. coli	-	-	-	8	14	-
P. mirabilis	-	-	-	8	25	-
P. vulgaris	-	-	-	8	18	-

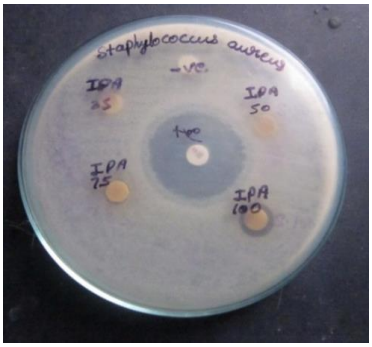
IPA : *Ipomea pes-caprae* aqueous extract



***Lactobacillus*. sp**



Bacillus subtilis

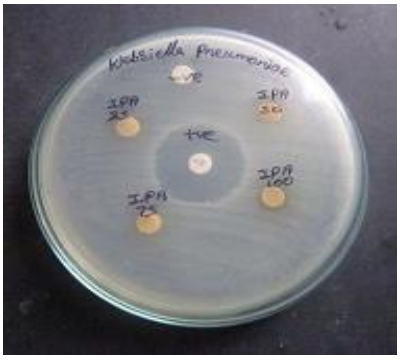


Staphylococcus aureus

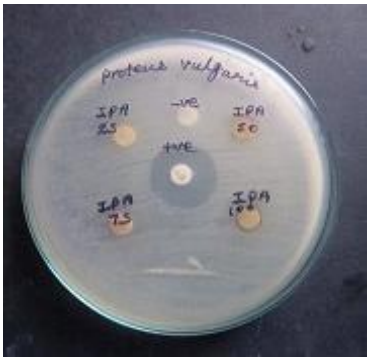
Streptococcus mutans

Figure 1: Antibacterial activity of aqueous extracts of flower *Ipomea pes-caprae* against human pathogenic gram positive bacteria

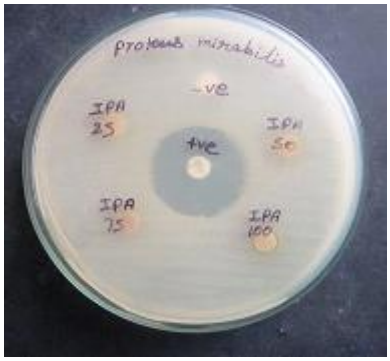
Escherichia coli



Klebsiella pneumoniae



Proteus vulgaris



Proteus mirabilis

Figure 2: Antibacterial activity of aqueous extracts of flower *Ipomea pes-caprae* against human pathogenic gram negative bacteria

Table 3: Percentage of cell viability and IC_{50} values of three cancer cell lines treated with aqueous extract of *Ipomea pes-caprae*

Cell lines	Percentage (%)					IC_{50} value µg/ml
	6.25	12.5	25	50	100	
HT 29	80.49	76.61	63.24	52.75	34.46	83.15
Mcf 7	85.82	68.45	62.02	60.99	29.51	75.15
A549	93.15	70.51	67.60	33.17	26.12	56.64

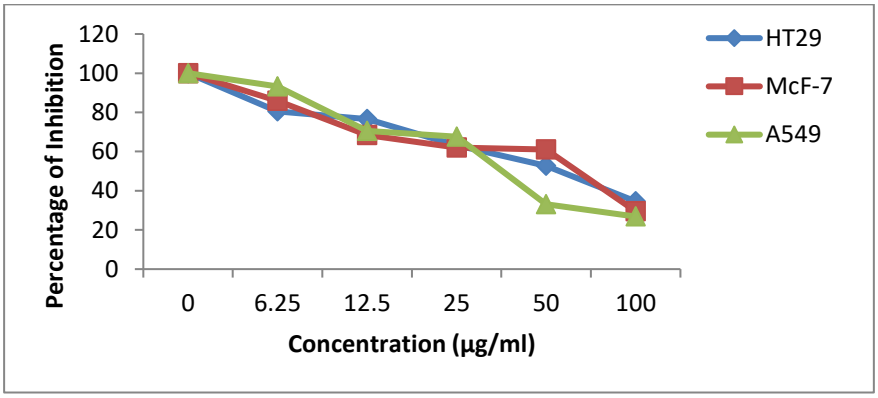


Figure 3: Percentage of cell viability of 3 cancer cell lines treated with various concentration of *Ipomoea pes-caprae.*

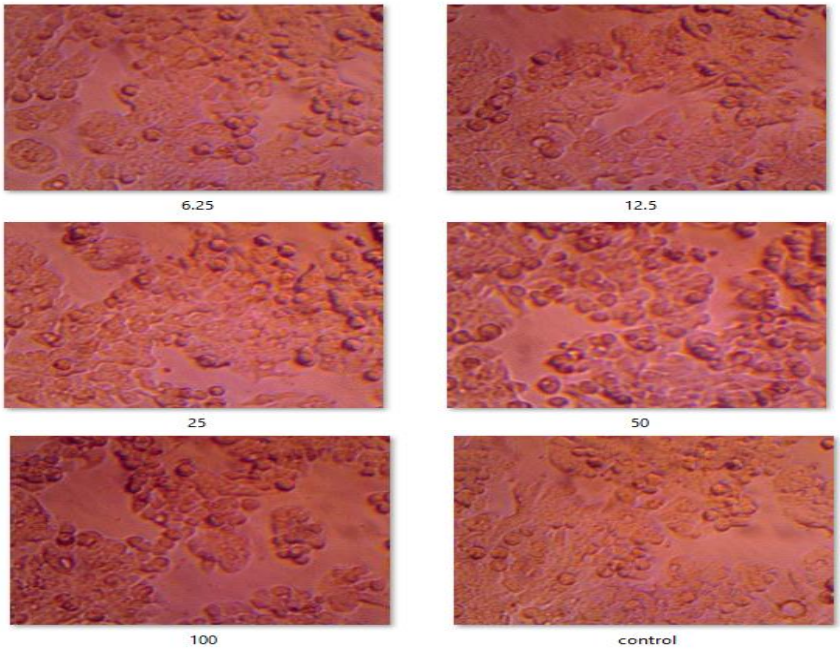


Figure 4: Microscopic image of HT29 cell lines treated with *Ipomoea pes-caprae* extract (µg/ml)

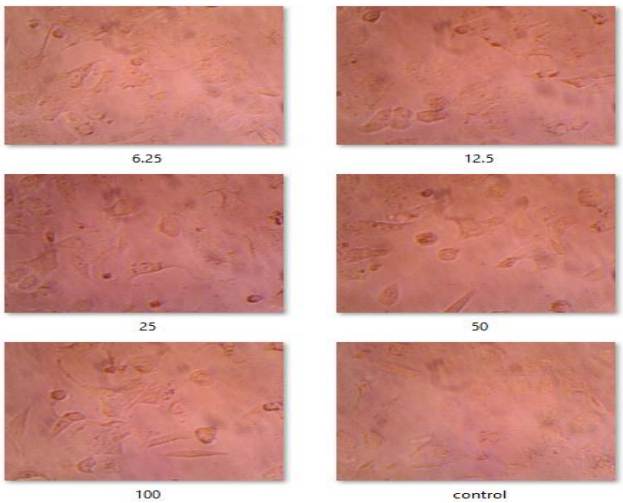


Figure 5: Microscopic image of MCF7 cell lines treated with *Ipomoea pes-caprae* extract (µg/ml)

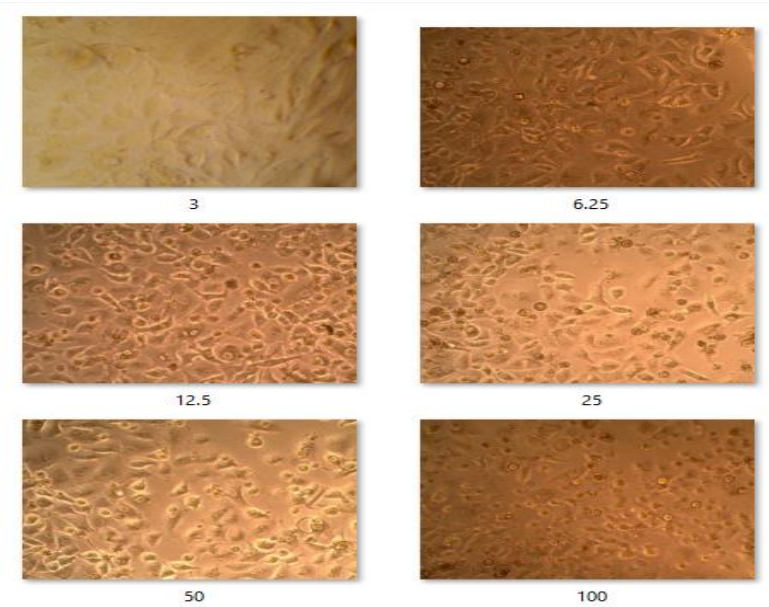


Figure 6: Microscopic image of A549 cell lines treated with *Ipomoea pes-caprae* extract (µg/ml)

CONCLUSION

The results obtained from the current study suggest that aqueous extracts of *Ipomea pes-caprae* flower contain good bioactive compounds and possess significant antibacterial and anticancer activity. Further purification of compounds and *in vivo* studies are under process.

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