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ANTIBACTERIAL EFFECT OF CRUDE ETHANOLIC LEAF EXTRACT OF *Ipomoea carnea* Jacq. ON *Pseudomonas aeruginosa*

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Ipomoea carnea, a native of South America, was introduced in India at the end of 19th century as a garden hedge plant. This belongs to the family Convolvulaceae. Phytochemical analysis of leaves exhibit presence of alkaloids (Calystegines and Swansonine), glycosides, phenolics and saponins. These secondry plant metabolites possesses antimicrobial activities. *Pseudomonas aeruginosa* is a Gram's negative, aerobic, rod shaped bacteria the causative agent of pneumonia, urinary tract infections (UTIs), bacteremia, wounds and burns. Screenning of crude ethanolic extract of leaves of *Ipomoea carnea* was done on *Pseudomonas aeruginosa* isolated from soil samples near J.P.N (Piligrim) Hospital Gaya. The bacteria was cultured on MacConkey agar medium. The MIC was carried out by agar well diffusion method. The crude ethanolic extract of the leaves of this plant inhibited the in vitro growth of Pseudomonas aeruginosa .

INTRODUCTION

Ipomoea carnea,a member of family Convolvulaceae, generally known as bush morning glory, is native of South America. In India it was introduced at the end of nineteenth century (Hooker, 1885) as garden and hedge plant and later on became common as weed.

The plant *Ipomoea carnea* is ever green flowering shrub which grows to a height of almost five metres. The stem is hollow and cylindrical in shape. The leaves are alternate, light green and cordate. The upper surface of leaf is dull green and lower surface is pailer. The plant is used as folk medicine in Ayurveda, Siddhaand Unani (Sharma and Bachheti, 2013). Antibacterial activity of crude extracts of the plant have been reported by various workers (Guleria and Kumar, 2006; Adsul *et al.*, 2012). The ethanolic leaf extract showed presence of hexadecanoic acid, stearic acid, 1,2 diethyl phthalate, octacosone, n-octadecanol, tetracontane and 3-diethylamino-1-propanol (Tirkey *et al.,* 1998). The presence of swainsonine and calystegines B1, B2, B3 and C1 were detected in the aquous ethanolic extract of leaf (Balogh *et al.,* 1999). Adsul *et. al.* 2012 reported antibacterial activity of ethanolic extract of *Ipomoea carnea* leaves on *Pseudomonas aeruginosa*.

P. aeruginosa is grams' negative rod shaped, free living, aerobic and bacterium which is prevalent in environment such as soils, seawater, sewage etc. It is highly versatile and adapt to various habitats. The bacteria cause many infectious diseases in humans such as UTIs, pneumonia, wounds and burns (Ullstrom 1991, Silby *et al.,* 2011).

Materials and Methods

1. **Collection of plant leaves :** *Ipomoea carnea* leaves were collected from Gaya town in the month of Oct. 2017. The leaves were thoroughly washed with water and rinsed with distilled water and dried in shade for two weeks and finally dried in thermostatic oven at a temperature not exceeding 30°C for 20 hours. The dried leaves were pulverized in a sterile electric blender to obtain fine powder and stored in air tight glass containers protected from sunlight.

2. **Isolation and Identification of *P. aeruginosa* from soil samples:** Soil samples were collected from J.P.N. (Piligrim) Hospital Gaya. Bacteriological analyses were conducted within four hours of samples collection. The soil sample was serially diluted and it was inoculated on sterilized petridishes containing MacConkey agar medium. The inoculated plates were shaken clockwise to make uniform distribution of inoculums. The plates were incubated at 370C for 24-48 hrs. After completion of incubation periods colonies were selected and identified on the basis of morphological and biochemical characteristics (Koneman *et al.,* 1998).

3. **Preparation of Crude ethanolic extract:** Ethanolic extract was prepared by using ethanol (99.5) by maceration method at room tempreture. 100gms of dried leaf powder was taken in round bottom flask, 500 ml of ethanol was poured and the mixture was kept under old maceration technique for 7 days with intermittent shaking. The extract was collected by filtering it through five layers of muslin cloth and concentrated at low temperature. The stock solution thus prepared was stored in desicators for further studies.

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4. **Phytochemical screening:** Ethanolic extract was subjected to qualitative phytochemical analysis for different secondary metabolites such as alkaloids, saponins, flavonoids, glycosides and tannins (Brain and Turner, 1975; Sofowora, 1993 and Edeoga et al., 2005).

5. **Antibacterial activity (Determination of Zone of inhibition of *P. aeruginosa*):** The antimicrobial activity of ethanolic extract of leaves of *Ipomoea carnea* was assyed by agar well diffusion method as described in NCCLS, 1993. Pertidishes containing 20 ml of nutrient agar medium was seeded with P. aeruginosa. Wells of approx. 10mm was bored in the plate containing NAM. Plant extracts were prepared in DMSO (Stock 1mg/ml DMSO). Plants extract of 25µl, 50 µl and 100 µl concentration were added and the plates were incubated at 37°C for 24 hrs. The secondary plant metabolites were allowed to diffuse in to the medium and they interacted with the *P. aeruginosa* bacterium and zone of inhibitions was measured in mm after 24 hours.

Table-1

Identification and characterization of Pseudomonas aeruginosa isolated from soil sample.

	Biochemical test	***Pseudomonas aeruginosa***
1	Gram's Staining	Gram Negative rods
2	Lactose Utilization test	- (No AG)
3	Indol production test	_
4	Methyl Red test	_
5	Voges Proskaur test	_
6	Citrate utilization test	+
7	Catalase test	+

(–) = Negative results, (+) = Positive Results, (AG) = Acid and Gas productions

Table-2

Phytochemical analysis of secondary metabolites

Sl.no	**Phytochemical test**	**Reagent used**	**observation**	**Result**
1	Alkaloid test	Dragendorff's test	Orange brown precipitate	+
2	Saponin test	Foam test	Froth formation	_
3	Flavonoid test	Lead acetic test	Yellow precipitate	+
4	Carbohydrate test	Benedict's test	Orange precipitate	+
5	Steroids test	Salwoski'test	Formation of Green colour	+
6	Tanin test	Gelatin test	Formation of Green colour	+

+ve sign indicates presence of secondary metabolites

-ve sign indicates absence of secondary metabolites

Table-3

Antimicrobial action of ethanolic extract of leaves of *Ipomoea carnea* Jacq using agar well diffusion method.

Different concentration of Ethanolic leaf extract		Diameter of zone of inhibition (in mm)
1	25 µl	10.62
2	50 µl	13.78
3	100 µl	17.18

OBSERVATIONS

The incubated MacConkey agar petriplates were examined for studying colony morphology where the colony appeared transparent and colourless. On microscopic examination done by Grams' staining process, the isolated bacteria were observed to be Gram (-) negative rods. The isolated bacterial colonies were biochemically examined. The isolates microorganisms showed positive results for citrate utilization test and catalase test (Table-1), whereas microorganisms showed negative results for lactose test, indol test and MR-VP test. All the above morphological and biochemical observations indicate the soil isolate as *P. aeruginosa*.

The preliminary qualitative phytochemical screening of ethanolic extract of leaves of *Ipomoea carnea* was performed and details of which are mentioned in Table-2. The antimicrobial action of ethanolic extract of leaves of Ipomoea carnea was performed by using three different concentration of leaves extract ie. 25µl, 50 µl and 100 µl agar well diffusion methods. The zone of inhibition for *P. aeruginosa* is given in Table-3.

DISCUSSION

In the present investigation colony morphology and biochemical chracterization were recorde which are similar to the findings of Buxton and Fraser (1997) Ali *et al.* (1998) and Su-swe-su *et al.* (2020). The biochemical chracterization of soil isolate was done by using Lactose fermentation test. Catalase test and IMViC test which was in accordance with Cheesbrough (1985). The isolate was identified as *P. aeruginosa* an oppourtunistic parasite causing infections in immune suppressed individuals. The phytochemical investigation of ethanolic leaf extract of *Ipomoea carnea* showed the presence of alkaloids, saponins, flavonoids and tannins etc.

The plant of *Ipomoea carnea* is repository of pharmalogically and medicinally bioactive phytoconstituents, therefore, this plant has shown tremendous application in traditional and modern medicine. In the current investigation the qualitative phytochemical analysis conducted has comprehensively established its therapeutic importance. The presence of various secondary metabolites acts synergistically to exhibit antibacterial properties. The alkaloids have antibacterial effects and saponins and glycosides showed cytotoxic potential (Apu *et al.,* 2020). Adsul *et al.* (2012) demostrated that ethanolic extract of leaves of the *I. carnea* showed promising inhibition against P. aeruginosa. A secondary metabolite dibutyl phthalate isolated from *I. carnea* exhibits antimicrobial activity (Khatiwora *et al.,* 2012). The present investigation revealed that the ethanolic crude leaf extract of the chosen plant has significant antibacterial activity against *P. aeruginosa* and antimicrobial property was established by using agar well diffusion method. The zone of inhibition gradually increased as the concentration (25µl, 50 µl and 100 µl) of plant extract was increased. Their inhibition was minimum at 10.62 mm for 25 µl extract where as at a concentration 100 µl, the zone of inhibition was 17.18 mm, which was significant. This finding was more or less in co-relation with the finding of Chaudhury *et al.* (2017). The antimicrobial property of ethanolic leaf extract can be a promising source in pharmaceutical industry as a potent antimicrobilal bioactive compound.

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