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RESEARCH ARTICLE

EFFECT OF *IPOMOEA PES-CAPRAE* (LINN.) R. BR. STEM AND ROOT EXTRACTS ON DIFFERENT CLINICAL BACTERIAL ISOLATES.

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Abstract

The aim of the present study was to investigate the antibacterial activity of chloroform, ethyl acetate, hexane and methanol extracts of *Ipomoea pes-caprae* (Linn.) R. Br. extracts of roots and stem of plant *Ipomoea pes-caprae* (Linn.) R. Br. The extracts were tested against different clinical bacterial isolates and the antibacterial activity was performed by using well-plate method. The results demonstrated that the methanol extract of *Ipomoea pes-caprae* stem and ethyl acetate extract of *Ipomoea pes-caprae* root exhibit strong antibacterial activity. Our results showed that this plant have observable therapeutic effects on potential infectious agents and can be used as an alternate to conventional medicines.

Introduction:-

The traditional medicine comprises several herbal and non-herbal constituents and it is hypothesized that they have potential to act on a number of targets through various mechanisms (Tiwari and Raos, 2002). It is observed that the herbal treatment options are associated with a small number of side effects in contrast to conventional ones (Sharma *et al.,* 2008). A critical problem coupled with the integration of medicinal herbs in advanced clinical practices is the inadequate amount of clinical and scientific data which suggested their safety and efficacy (Modak *et al.,* 2007).
Indiscriminate use of antibiotics is a major factor responsible for the emergence and dissemination of multidrug resistance strains of microorganisms. This urges the researchers to move their attention towards herbal products research (Khan *et al*., 2009). The use of plant-based therapies is increasing in both underdeveloped and developed countries. As they are natural products, having affordable prices, less toxicity, no deleterious side effects, are simply biodegradable and are easily accessible (Aruna and Nandakishore, 2014).

Humans are the important host of a number of bacterial pathogens, cause several infectious diseases. *Proteus mirabilis* has an ability to cause empyema and osteomyelitis (Bahashwan and Shafay, 2013). *Shigella dysenteriae* cause serious infections because of the production of shiga toxin (Omololu-Aso *et al.,* 2017). *Klebsiella* cause meningitis, bacteremia, urinary tract infections and pneumonia (Kumar *et al.,* 2016; Jayaraj *et al.,* 2014). According to the literature, about 250,000 people died per year globally because of the typhoid fever which is caused by

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Salmonella typhi (Saleh *et al.,* 2014). *Salmonella typhi para A* cause enteric fever usually known as paratyphoid (Naveed and Ahmed, 2016). *Escherichia coli* is associated with urinary tract infections mainly cystitis (Kariuki *et al.,* 2007) and *Enterococcus faecalis* is now ranked among the top three nosocomial bacterial pathogens (Kayaoglu and Orstavik, 2004). *Pseudomonas aeruginosa* is responsible for causing pneumonia and also severely affects the lungs of patients suffering from cystic fibrosis (Debarbieux *et al.,* 2010). *Staphylococcus epidermidis* can cause cojunctivitis and endophthalmitis (Dave *et al.,* 2011). *Staphylococcus saprophyticus* is a causative agent of urinary tract infections (UTIs) in young and adult females (Raz *et al.,* 2005; Widerstrom *et al.,* 2012). Methicillin resistant *Staphylococcus aureus (MRSA)* cause infections associated with high mortality rate including nasal infections (Parasa *et al.,* 2011; Chao *et al.,* 2008).

The genus *Ipomoea* belongs to the family Convolvulaceae. It is the largest genus with about 500 species distributed throughout the tropics and subtropics of both the hemispheres. *Ipomoea pes-caprae* (Linn.) R. Br. is a climber with its stem trailing and rooting at the nodes and the stem is usually glabrous and hard. This is a halophytic plant found abundantly in the coastal areas. Its common name is "Samudraphen". The plant is used in medicine and the leaves are applied externally in rheumatism and colic (Austin and Ghazanfar, 1979).

In this study, the antimicrobial effect of different extracts was observed on various gram positive and gram negative clinical bacterial isolates including *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhi para A, Klebsiella pneumoniae, Citrobacter species, Enterobacter species, Methicillin-resistant Staphylococcus aureus (MRSA), Shigella dysenteriae, Streptococcus fecalis, Staphylococcus epidermidis* and *Staphylococcus saprophyticus*.

Materials and Method:-

Collection of Herbs:

The root and stem parts of *Ipomoea pes-caprae* (Linn.) R. Br. were collected from coastal area of Karachi and the voucher specimen has been submitted in the Karachi university herbarium (G.H.no.86616). The plant was identified by the taxonomist in Herbarium, Center for Plant Conservation which is situated in the University of Karachi, Karachi.

Collection of clinical bacterial isolates:

The clinical bacterial isolates were collected from patients sample and identified by using the standard microscopical, colonial and biochemical characteristics. The bacterial isolates were collected from a diagnostic laboratory.

Preparation of Herbal Extracts:

For the preparation of herbal extracts, the roots and stem parts of *Ipomoea pes-caprae* (Linn.) R. Br. were washed, dried, crushed and grinded to convert into powdered form. The soxhlet apparatus was used to make different extracts of the collected plant. 15 grams of powdered plant material was weighed and wrapped in whatsman 41 filter paper. The plant material was placed inside the extraction tube, over which a condenser was fixed. The soxhlet apparatus was connected to a distillation flask and a chiller which was set at temperature 5˚C. 150 ml of respective solvent i.e. hexane, chloroform, ethyl acetate and methanol was poured respectively in the distillation flask placed on a heating mantle. The temperature was adjusted in accordance with the solvent used (30˚C-40˚C). The extraction procedure was continued for about 14-15 hours. The extract was then transferred to a round bottom flask to concentrate by using BUCHI Rota-vapour R-200. The flask containing the extract sample was submerged in a water bath set to the temperature 40˚C. The concentrated extract was partitioned in the extract tube and left opened for the removal of any residual solvent. The dried form of extract was kept at 4˚C for experimental procedures (Redfern *et al.,* 2014).

Effect of plant extracts on clinical bacterial isolates:

For the preparation of plant extracts, different solvents were used including methanol, ethyl acetate, hexane and chloroform. DMSO was used to make different concentrations of four types of extracts i.e. 250µg, 500µg, 750µg, 1000µg, 5000µg. Extract concentrations were selected after performing MIC assay.

Preparation of lawns:

Mueller Hinton agar was used to see the antibacterial activity by performing well-diffusion technique. 0.5 McFarland's index was prepared to get the inoculum size of 1.5×10^8CFU/ml (Coyle, 2005). 0.1ml was added and

lawns were prepared by the help of spreader. After certain period of time, wells were made on each plate with the help of a borer. 50µl of each extract concentrations i.e. 250µg, 500µg, 750µg, 1000µg, 5000µg, was added in the respective wells. 50µl of DMSO was added in one well as a negative control and the plates were incubated for 24 hours.

Measurement of zone of inhibition:
After incubation, the plates were observed for the zone of inhibition around the wells and the diameter was measured in millimetres (mm).

Statistical analysis:-

Statistical analysis was performed by using the software IBM SPSS Statistics 23. One way analysis of variance (ANOVA) followed by Bonferroni post hoc test and student's t-test were performed to compare the groups with level of confidence $P < 0.05$; (where * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$). Data are presented as mean ± SEM.

Results:-

In this study, the antimicrobial effect of various extracts was observed on thirteen clinical bacterial isolates including *Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, Salmonella typhi para A, Klebsiella pneumoniae, Citrobacter species, Enterobacter species, Methicillin-resistant Staphylococcus aureus (MRSA), Shigella dysenteriae, Streptococcus fecalis, Staphylococcus epidermidis* and *Staphylococcus saprophyticus.* We used clinical isolates of bacteria to ensure that these strains are pathogenic.

All the clinical bacterial isolates were treated with five concentrations of hexane extract of *Ipomoea pes-caprae* stem i.e. 250µg, 500µg, 750µg, 1000µg and 5000µg (Fig. 1a). In case of *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter species, Salmonella typhi para A, Staphylococcus saprophyticus, Enterobacter species, Klebsiella pneumoniae* and *Methicillin-resistant Staphylococcus aureus (MRSA),* statistically significant and large zones of inhibition were produced as compared to the control, showing that this extract possesses high activity against these organisms ($p < 0.001$***). No zone of inhibition was found at any concentration of the extract when *Shigella dysenteriae, Streptococcus fecalis* and *Escherichia coli* were exposed to this treatment.

In the same manner, when bacterial isolates were treated with different concentrations of chloroform extract of *Ipomoea pes-caprae* stem (Fig. 1b), it was observed that clear zones of inhibition were present around all the wells of *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter species, Enterobacter species, Klebsiella pneumoniae* and *Salmonella typhi para A*. This shows that these organisms are highly sensitive against the chloroform extract while *Staphylococcus saprophyticus* and *Shigella dysenteriae* were insensitive to the lower concentrations of the extract. Only one organism i.e. *Methicillin-resistant Staphylococcus aureus (MRSA),* showed non-significant results as small zones were produced. This extract showed no activity against *Streptococcus fecalis, Escherichia coli* and *Staphylococcus epidermidis.*

When we analyzed the effect of ethyl acetate extract of *Ipomoea pes-caprae* stem (Fig. 1c) on the selected clinical bacterial isolates, the results indicated that this extract exhibits significant antibacterial activity against *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter species, Enterobacter species, Klebsiella pneumoniae, Salmonella typhi para A, Staphylococcus epidermidis, Methicillin-resistant Staphylococcus aureus (MRSA), Shigella dysenteriae* and *Escherichia coli* as significant zones were appeared ($p < 0.001$***). In case of *Staphylococcus saprophyticus,* non-significant zones were found. *Streptococcus fecalis* was able to survive at all the concentrations of the extract. The control did not show any antibacterial activity.

The results of methanol extract of *Ipomoea pes-caprae* stem (Fig. 1d) showed that this extract significantly ($p < 0.001$***) affect the growth of *Salmonella typhi, Pseudomonas aeruginosa, Citrobacter species, Enterobacter species, Klebsiella pneumoniae, Salmonella typhi para A, Staphylococcus saprophyticus, Shigella dysenteriae* and *Methicillin-resistant Staphylococcus aureus (MRSA).* This extract showed non-significant activity against *Proteus mirabilis* and *Staphylococcus epidermidis*. *Escherichia coli* and *Streptococcus fecalis* remained insensitive to all the concentrations as no zone of inhibition was found.

To evaluate the effect of hexane extract of *Ipomoea pes-caprae* root (Fig. 2a), same method was followed. When the clinical bacterial isolates were treated with five different concentrations of the extract, i.e. 250µg, 500µg, 750µg,

1000µg and 5000µg, it was observed that *Proteus mirabilis, Citrobacter species, Salmonella typhi para A, Pseudomonas aeruginosa, Enterobacter species* and *Klebsiella pneumonia* were significantly sensitive to this extract ($p < 0.001$***). Non-significant zones were produced in case of *Salmonella typhi*. However, this extract was unable to inhibit the growth of *Staphylococcus saprophyticus, Methicillin-resistant Staphylococcus aureus (MRSA), Shigella dysenteriae, Streptococcus fecalis* and *Escherichia coli.*

Moreover, when the chloroform extract of *Ipomoea pes-caprae* root (Fig. 2b) was examined, it was observed that few organisms i.e. *Salmonella typhi, Proteus mirabilis, Citrobacter* and *Salmonella typhi para A* were significantly inhibited by all the concentrations of this extract ($p < 0.001$***). *Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter species, Staphylococcus saprophyticus, Methicillin-resistant Staphylococcus aureus (MRSA), Shigella dysenteriae, Staphylococcus epidermidis, Streptococcus fecalis* and *Escherichia coli* showed no sensitivity to this extract.

When we used the ethyl acetate extract of *Ipomoea pes-caprae* root (Fig. 2c), the statistically significant difference was observed between the zones of inhibition of the treated and the control wells. In case of *Salmonella typhi, Shigella dysenteriae, Salmonella typhi para A, Shigella dysenteriae, Citrobacter species, Staphylococcus saprophyticus, Staphylococcus epidermidis* and *Streptococcus fecalis*, significant zones were observed ($p < 0.001$***). Non-significant zones appeared in case of *Proteus mirabilis, Pseudomonas aeruginosa and Enterobacter species* and no zone was observed at any concentration in case of *Klebsiella pneumoniae* and *Escherichia coli.*

Methanol extract of *Ipomoea pes-caprae* root (Fig. 2d) showed significant activity against *Salmonella typhi, Pseudomonas aeruginosa, Citrobacter species, Shigella dysenteriae, Staphylococcus saprophyticus, Escherichia coli* and *Salmonella typhi para A* ($p < 0.001$). This shows that these organisms were significantly sensitive against the methanol extract while *Enterobacter species, Streptococcus fecalis, Methicillin-resistant Staphylococcus aureus (MRSA)* and *Staphylococcus epidermidis* were insensitive to the lower concentrations of the extract and showed significant zones at 5000µg concentration only ($p < 0.001$***). Only one organism *Proteus mirabilis* showed non-significant zone of inhibition while no effect on *Klebsiella pneumoniae*.

Discussion:-

In this study, different solvents were used due to the fact that these organic solvents possess variable polarity for different phyto-compounds. On the basis of number of organisms that showed sensitivity, the order of the inhibitory potential of the stem extracts of *Ipomoea pes-caprae* against the clinical bacterial isolates was observed as ethyl-acetate extract > methanol extract > chloroform and hexane extract. Similarly, in case of root extracts of *Ipomoea pes-caprae*, the order of inhibitory potential was methanol extract > ethyl-acetate extract > hexane extract > chloroform extract.

Collectively, the results shown in Table.1 demonstrate that the methanol extract of roots and the ethyl acetate extract of stem were more effective against all the tested clinical bacterial isolates as maximum number of bacteria showed susceptibility against these extracts. However, considerable larger zones of inhibition were observed in case of hexane and chloroform extracts of stem. Among our tested clinical isolates, *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter species*, and *Salmonella typhi para A* showed sensitivity to all the extracts of *Ipomoea pes-caprae* whereas *Escherichia coli* and *Streptococcus fecalis* showed sensitivity to the least number of extracts. In this view, chloroform extract of stem was able to produce the largest zone of inhibition of diameter 26mm in *Salmonella typhi para A*. On the other hand, same extract produced smallest zone of inhibition in case of *Methicillin-resistant Staphylococcus aureus (MRSA).*

The antibacterial activity of these extracts might be due to the compounds like pes-caprein, sterols and other phytochemicals which can be dissolved in various organic solvents. The lower inhibitory potential of some extracts might be due to the fact that some compounds are volatile in nature or get denatured in *in-vitro*. On the other hand, it might be possible that some organisms did not allow the entrance of the extract inside the cell or those bacteria were insensitive to a particular compound present in that extract (Gull *et al*., 2012).

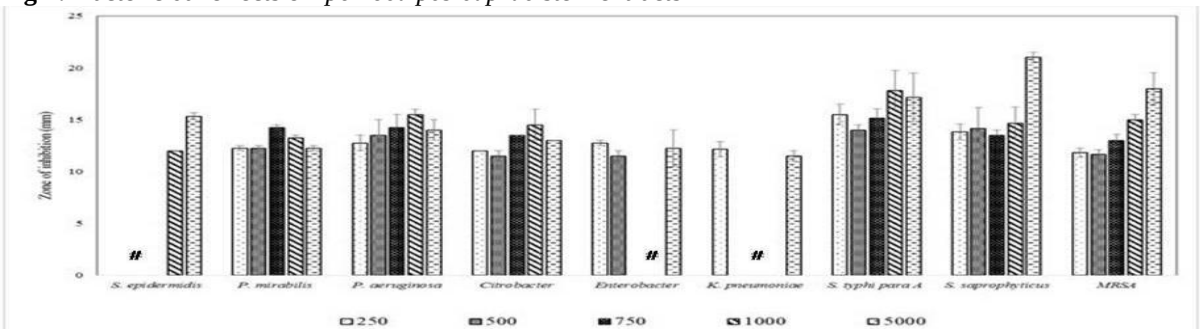
Conclusion:-

Our results demonstrate that the chloroform and ethyl acetate extracts of stem of *Ipomoea pes-caprae* and methanolic extract of roots of *Ipomoea pes-caprae* showed strong inhibitory action against almost all tested clinical isolates. The present study may provide an evidence of the therapeutic potential of *Ipomoea pes-caprae*. Our results support the use of the aforementioned plant against *Salmonella typhi, Proteus mirabilis, Pseudomonas aeruginosa, Citrobacter species* and *Salmonella typhi para A* infections.

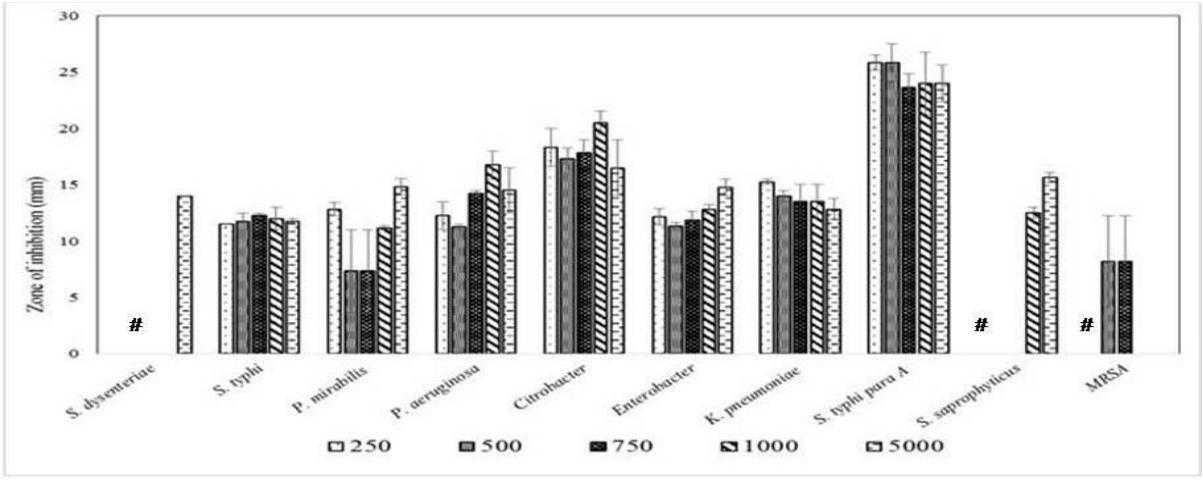
Table I:-Highest zone of inhibition (mm) against different clinical bacterial isolates at variable Extracts' concentrations

Clinical Isolates	***Ipomoea pes-caprae* STEM (zone of inhibition, in mm)**				***Ipomoea pes-caprae* ROOT (zone of inhibition, in mm)**			
	Hexane Extract	**Chloroform Extract**	**Ethyl acetate Extract**	**Methanol Extract**	**Hexane Extract**	**Chloroform Extract**	**Ethyl acetate Extract**	**Methanol Extract**
Salmonella typhi	18	12	16	14	11	14	14	15
Proteus mirabilis	14	15	13	9	18	14	8	15
Pseudomonas aeruginosa	16	17	13	13	12	0	12	12
Citrobacter species	15	21	14	14	13	12	12	12
Enterobacter species	13	15	12	12	12	0	13	12
Klebsiella pneumoniae	12	15	12	13	12	0	0	0
Salmonella typhi para A	18	26	15	11	12	12	14	13
Staphylococcus saprophyticus	14	16	8	18	0	0	14	15
Methicillin Resistant Staphylococcus aureus (MRSA)	18	8	12	11	0	0	15	13
Staphylococcus epidermidis	15	0	15	8	0	0	13	15
Escherichia coli	0	0	11	0	0	0	0	13
Streptococcus fecalis	0	0	0	0	0	0	13	13
Shigella dysenteriae	0	14	13	15	0	0	13	12

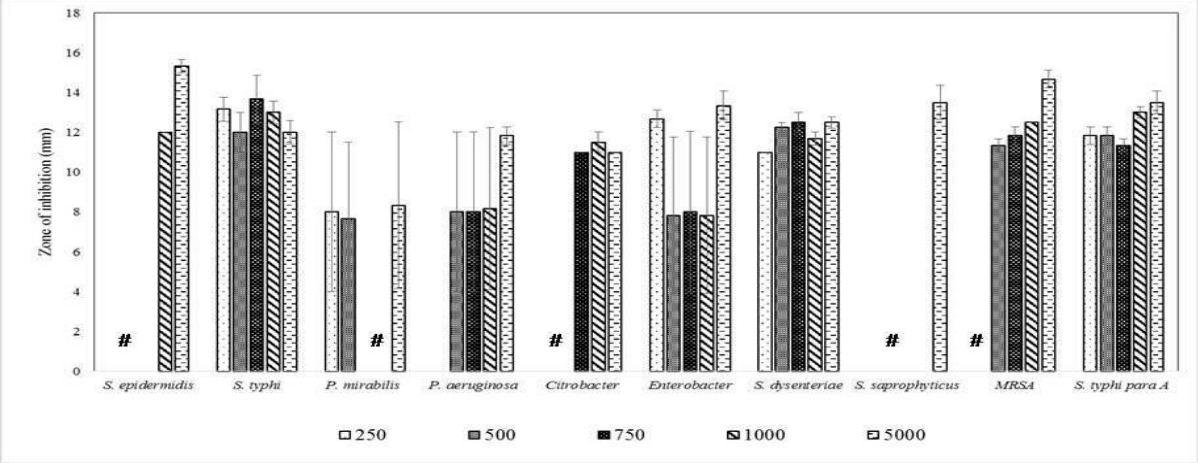
Fig 1:-Bactericidal effects of *Ipomoea pes-caprae* stem extracts



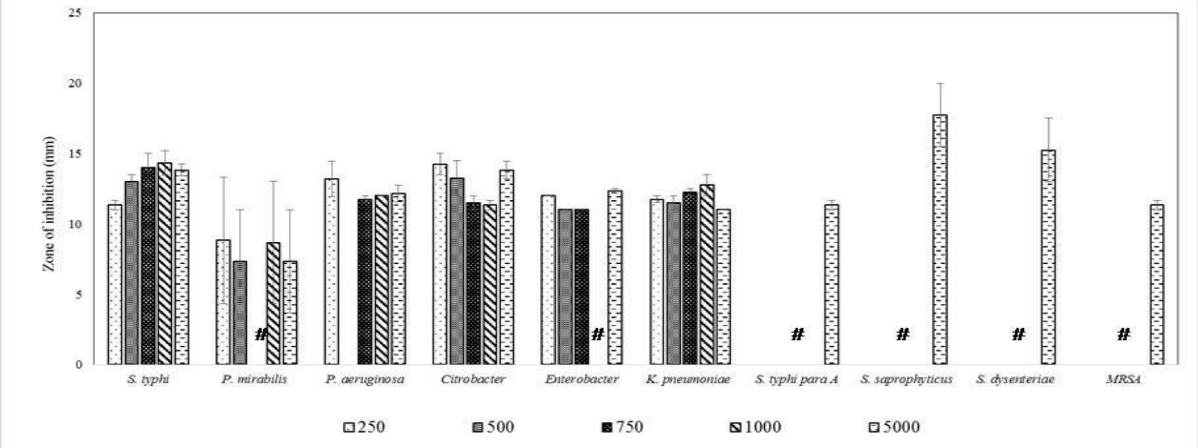
(a) Hexane extract, # shows no zone of inhibition



(b) Chloroform extract, *# shows no zone of inhibition*

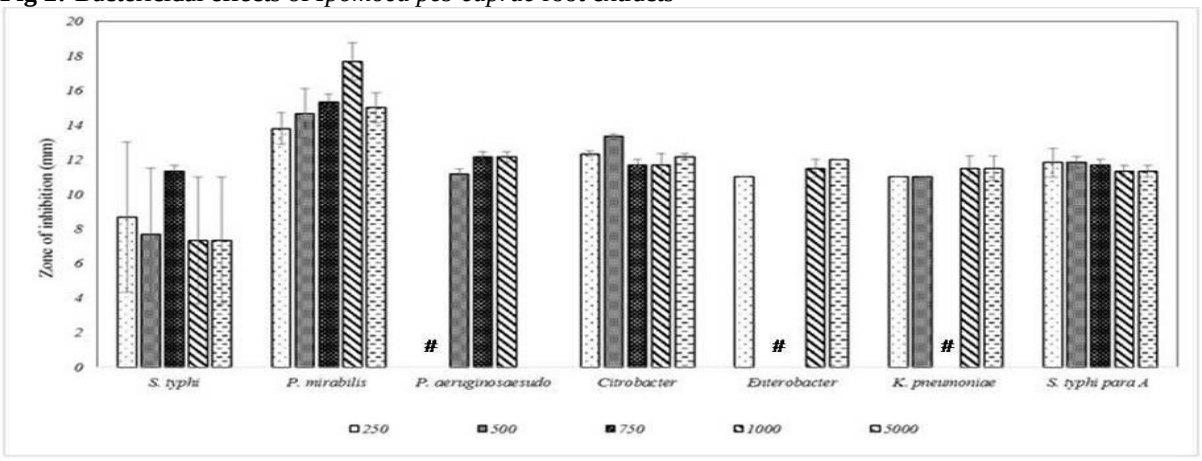


(c) Ethyl acetate extract, *# shows no zone of inhibition*

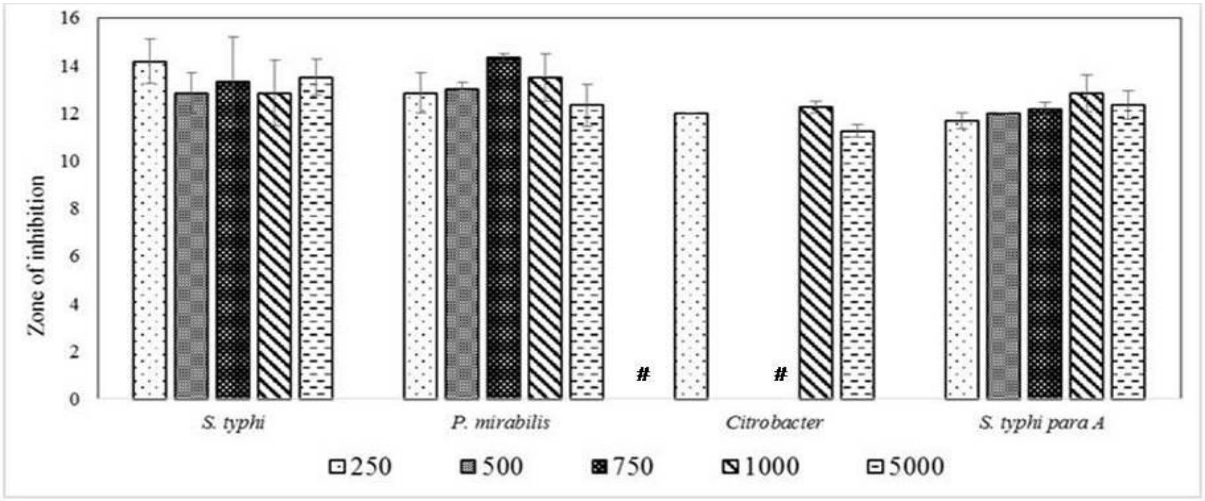


(d) Methanol extract, *# shows no zone of inhibition*

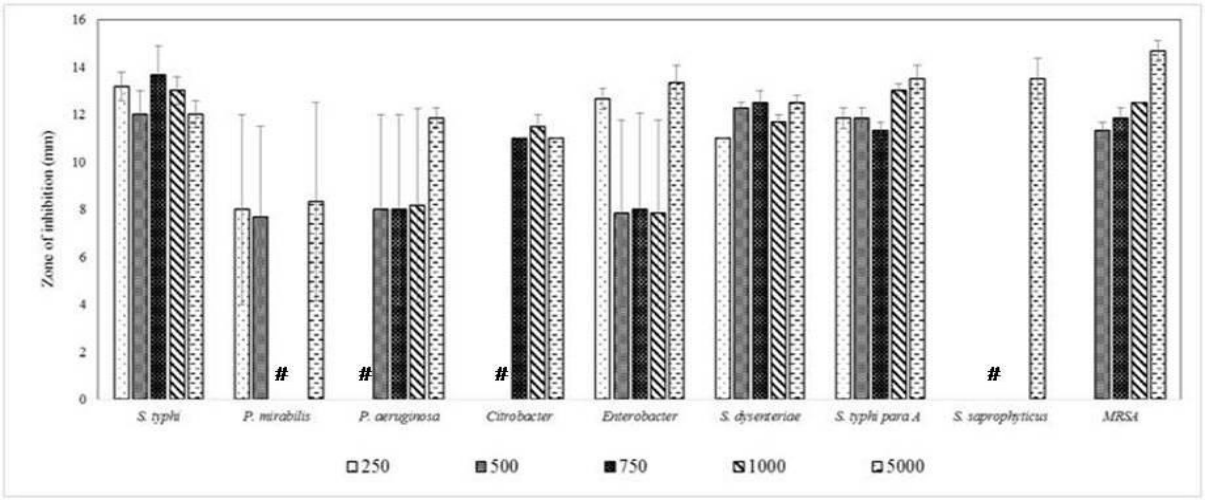
Fig 2:-Bactericidal effects of *Ipomoea pes-caprae* root extracts



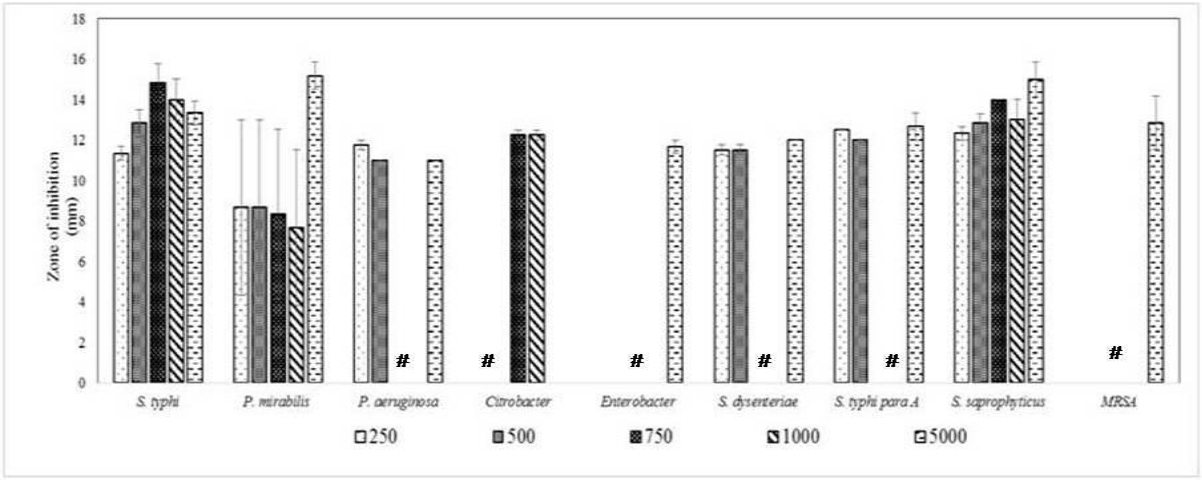
(a) Hexane extract, # shows no zone of inhibition



(b) Chloroform extract, # shows no zone of inhibition



(c) Ethyl acetate extract, # shows no zone of inhibition



(d) Methanol extract, # shows no zone of inhibition

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