ORIGINAL RESEARCH

EVALUATION OF AN HERBAL ANTISEPTIC DRUG DELIVERY SYSTEM

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ABSTRACT:

Background: In ano-rectal surgery, *Ayurvediya* surgeons*use kshar-sutra*,for the surgical management of piles, fistula- in- ano, etc., which is formulated with medicinal herbs. But to avoid infections during these procedures, surgeons have to use allopathic topical anti-septic formula or depend on the inherent immunity of the patients to prevent infections.

Objective: Both the plants i.e. *Shigru* & *Ingudi* taken for study are well known in Ayurveda for their *krimighna* action. Therefore this study was carried out to contemplate an herbal anti-septic formula against infection causing organisms to guard the surgical wounds.

Methods: Both aqueous and alcoholic extracts of individual samplesat 10, 20, 30% concentrations & mixed solutions at 30% concentrations were tested for antimicrobial activity by agar well diffusion method against a range of gram-positive and gram-negative bacteria. Zone of inhibition of extracts & activity index were calculated.

Results: Ethanolic extract of root bark of *Moringa oleifera* Lam. has more potent action against all microorganisms with ZOI (12, 16, 11, 13mm respectively). Aqueous extract of *Balanites aegyptiaca* Linn. Delile has more potent action against microorganism *Staphylococcus aureus, Escherichia coli, Klebsiella aerogenes,* ZOI (15,11, 19mm respectively). Ethanolic extract of all three samples showed results on *Escherichia Coli, Klebsiella aerogenes* with zone of inhibition of 10mm & with activity index of 0.6, 0.75 & 0.52 respectively.

Conclusion: Individual drugs showed significant effects on all four bacterial strains. But they show dissimilar inhibitory response due to difference in plant metabolites. Decoction or paste of individual drugs can be used for washing or local application on surgical wounds but mixture should be avoided as it will not show significant results.

KEYWORDS: *Shigru, Ingudi, Krimi,* Antimicrobial activity, Zone of inhibition.

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INTRODUCTION:

Infectious diseases have long been a major health concern to entire human population, more in under developed & developing countries of the world like India. Infections occurring during & after surgery are also a major problem for already suffering patients, which can even cost life of the patient or make him/her physically disturbed. Although with the advent of antibiotics, many different bacteria & diseases caused by them have been controlled. But with every passing day these antibiotics are rendered ineffective due to resistance developed in microbes. These antibiotics have many toxic effects not only against microbes but also to the human being themselves. To over-come this, research is on for finding a drug which is effective against the diseases causing pathogens & at the same time does not produce toxic effect.

In ano-rectal surgery, *kshar-sutra*, which is highly effective preparation for the surgical management of piles, fistula- in- ano, etc., is formulated with the help of medicinal herbs. But even during these surgical procedures, *Ayurvediya* surgeons either have to use allopathic topical anti-septic formula or have to depend on the inherent immunity of the patients to prevent infections in the surgical wounds. Therefore this study was carried out to find out a herbal anti septic formula to prevent from infections during & after surgical infections.

For this study, leaves of *Ingudi (Balanites aegyptiaca* (Linn.) Delile*)*, leaves & root bark of *Shigru (Moringa oleifera* Lam.*)* were taken for the anti-microbial study.Different antimicrobial studies have rendered these individual drugs effective against various strains of bacteria. But antimicrobial effect of these combined drugs as a whole is still to be assessed.

Fig 1.1 of *Shigru* leaves

Fig 1.2 of *shigru* root bark

Fig 1.3 of *Ingudi* leaves & fruits

MATERIAL & METHODS:

Plant material

Leaves & root bark of *Shigru* & *Ingudi* were collected from *Jhag* village, *Dudu* tehsil, *Jaipur* & were authentified at herbarium section, Department of Botany, Rajasthan University, Jaipur with authentication no. RUBL211520 & RUBL 211519 respectively.

Preparation of extracts

Cold maceration method was used for preparing extracts. Macerated 10 g of the air dried drug, coarsely powdered, with 200 ml of solvent i.e. for preparing ethanol extract, ethanol solvent was used & for preparing aqueous extract, distilled water was used. Procedure was done for the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowed to stand for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, evaporated the filtrate to dryness in a tarred flat bottomed shallow dish, and dried at 105^0C, to constant weight& weighed[i]. The yield was 15.1% for ethanol extract of leaves of *Moringa oleifera* Lam.,23.26% for aqueous extract of leaves of *Moringa oleifera* Lam., 8.8% for ethanol extract of root bark of *Moringa oleifera* Lam., 11.84% for aqueous extract of root bark of *Moringa oleifera* Lam.21.58% for ethanol extract of

Balanitesa egyptiaca (Linn.) Delile & 19.94% for aqueous extract of *Balanitesa egyptiaca* (Linn.) Delile. Repeated the procedure for 5 times, so as to collect enough amount of extract required for antimicrobial activity. For carrying out antimicrobial activity, 10%, 20%, 30% concentrations of both ethanol & aqueous extracts were prepared by dissolving them in ethanol/water.

Antimicrobial activity

Micro-organisms- Bacterial strains selected for the study were *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Klebsiella aerogenes.* Out of which *Pseudomonas aeruginosa, Escherichia coli, Klebsiella aerogenes* are gram negative bacteria, whereas *Staphylococcus aureus* is gram positive bacteria. Reason for selecting these strains was, they are causative organism for surgical wound infections & mostly causes infections in piles, fistula in ano etc.

The pathogenic strains of above bacteria were procured from 'Institute of Microbial Technology' (IMTECH), Chandigarh and the stock cultures maintainance & antibacterial study was done at 'microbiology lab, *Dravyaguna Vigyan* Deptt, NIA', Jaipur.

MTCC No. 39-*Klebsiella aerogenes*

MTCC No. 10239- *Escherichia coli*

MTCC No. 1034- *Pseudomonas aeruginosa*

MTCC No. 6908- *Staphylococcus aureus*

Revival of microbial cultures

Microbes collected from Institute of Microbial Technology were in dried form. It needed to be revived.

Dried & freezed bacteria were revived by transferring them to conical flasks with nutrient broth media, kept at 37^0C to get cultures.

Preparation of media & media plates

Muller-hington agar medium was taken for all pathogens. 38 gram of agar was dissolved in 1 litre of distilled water. Heated the agar with water at 100^0C till it becomes transparent, then kept it in hot air oven for 15 minutes. The sterilized media were poured in sterile petri dishes aseptically. The Agar (solidifying agent), which was added in a broth medium, hardens at it cools. After solidifying of agar plates (nearly about 15 to 20 minutes), they were kept inverted in incubator at 37ºC for overnight for checking any contamination. The agar plates were ready.

Applied a microbial culture to the surface in a petri plate and spread them with cotton swab sticks. The prepared plates were then incubated in inverted position at 37ºC for 24 hours. After incubation, we got the pure cultures. This procedure is termed as 'Sub culturing'. In this way, frequent sub-culturing was done whenever required during antibacterial study.

Well diffusion method

For bactericidal assay in vitro, well diffusion method was adopted[ii], because of reproducibility and precision. Wells (of about 4mm diameter) were made on the plates with the help of sterile stainless steel borer. About 20-30µl different concentrations of plant solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs [iii] .Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 48 hours for bacterial pathogens. The zone of inhibition were measured around sterilized wells (4 mm in diameter). The 4 readings were taken in different planes, and then the mean was calculated.

Group design

Test group- 10%, 20%, 30% concentrations of ethanol extracts of leaves of*Balanitesa egyptiaca* (Linn.) Delile.

10%, 20%, 30% concentrations of aqueous extracts of leaves of *Balanitesa egyptiaca* (Linn.) Delile.

10%, 20%, 30% concentrations of ethanol extracts of leaves& root bark of*Moringa oleifera* Lam.separately

10%, 20%, 30% concentrations of aqueous extracts of leaves & root bark of*Moringa oleifera* Lam. separately

Standard group- 5% w/v povidine-iodine solution

Negative control group- Distilled water & Ethanol

Determination of activity index

The activity index of the crude plant extract was calculated as[iv]:

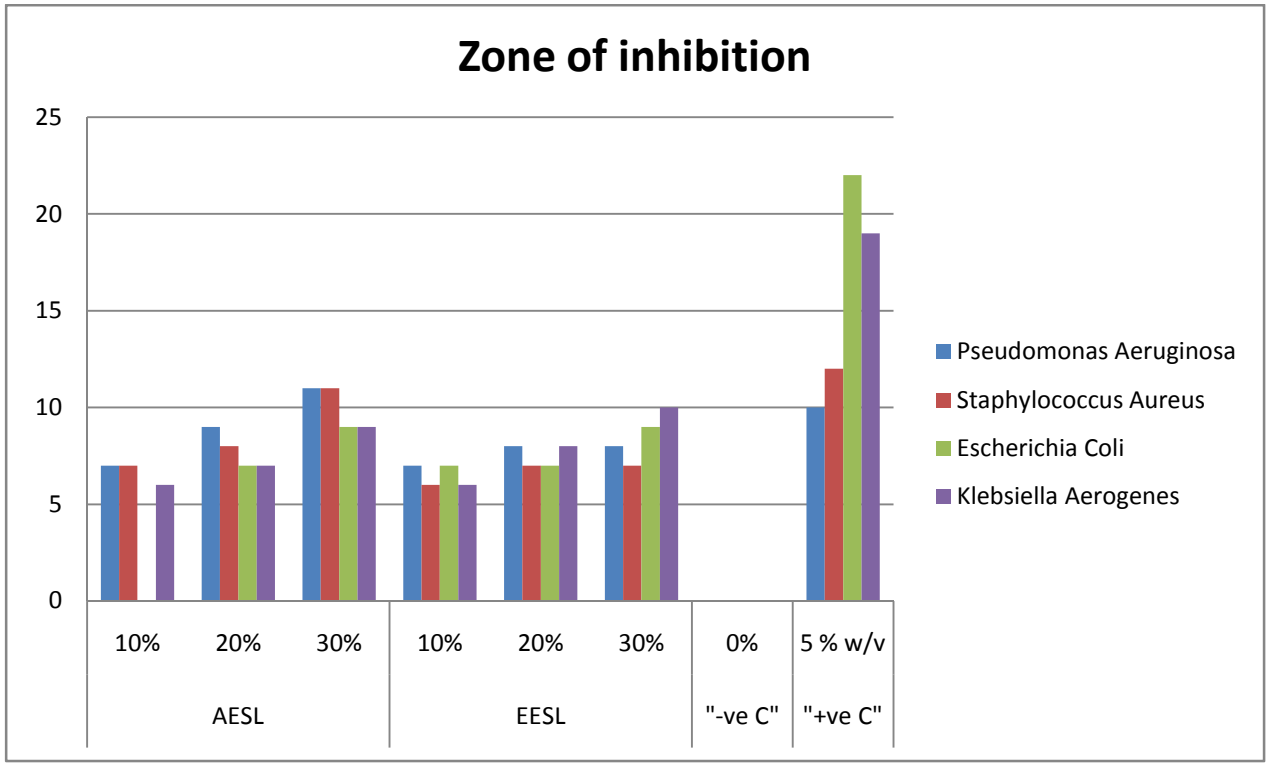
Activity index (A.I.) = Mean of zone of inhibition of the extract/ Zone of inhibition obtained for standard antibiotic drug

RESULTS:

***Shigru* leaf :**

Bacteria	Zone of Inhibition (in mm)							
	AESL			EESL			-ve Control	+ve Control
	10 %	20 %	30 %	10 %	20 %	30 %	0 %	5 % w/v
Pseudomonas aeruginosa	7	9	11	7	8	8	0	10
Staphylococcus aureus	7	8	11	6	7	7	0	12
Escherichia coli	0	7	9	7	7	9	0	22
Klebsiella aerogenes	6	7	9	6	8	10	0	19

Table 1.1: Showing zone of inhibition of *Shigru* leaves against four bacterial strains where AESL is aqueous extract of *shigru* leaves, EESL is ethanolic extract of *shigru* leaves

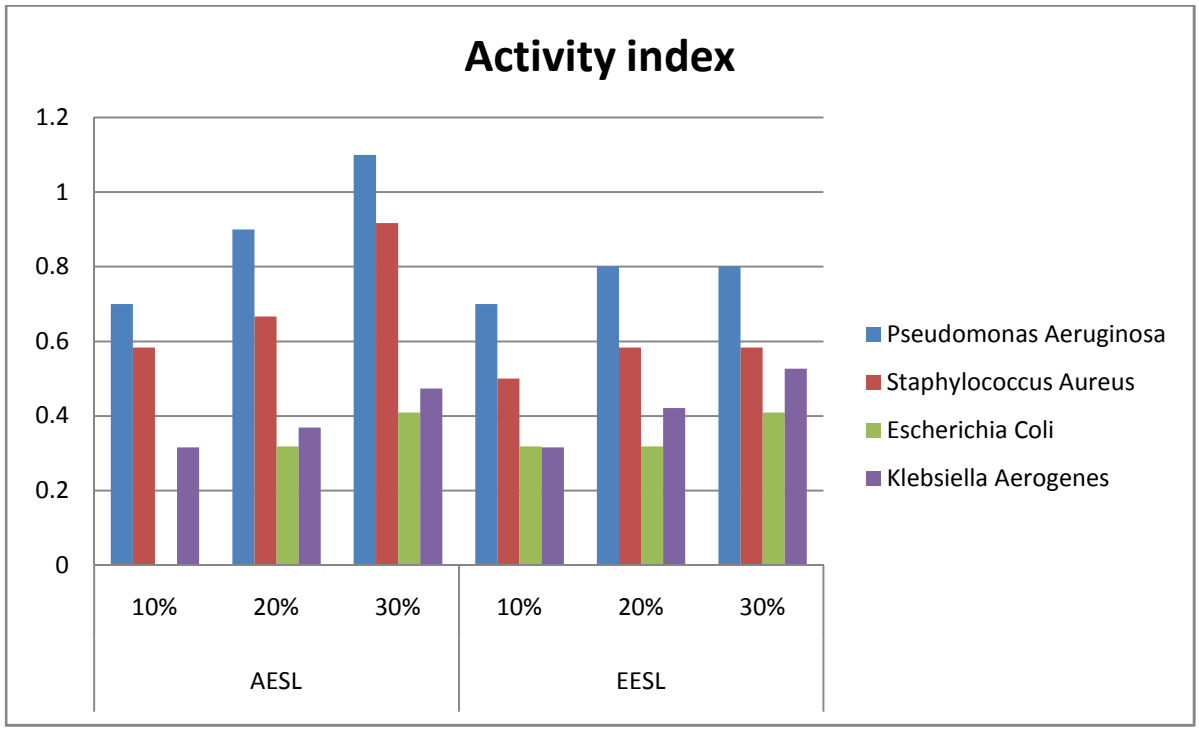


Graph 1.1: Showing zone of inhibition of *Shigru* leaves against four bacterial strains

Activity index:

Bacteria	Zone of Inhibition (in mm)					
	AESL			EESL		
	10%	20%	30%	10%	20%	30%
Pseudomonas aeruginosa	0.7	0.9	1.1	0.7	0.8	0.8
Staphylococcus aureus	0.58	0.67	0.91	0.5	0.58	0.58
Escherichia coli	0	0.32	0.41	0.32	0.32	0.41
Klebsiella aerogenes	0.31	0.37	0.47	0.31	0.42	0.52

Table 1.2: Showing Activity index of *Shigru* leaves against four bacterial strains

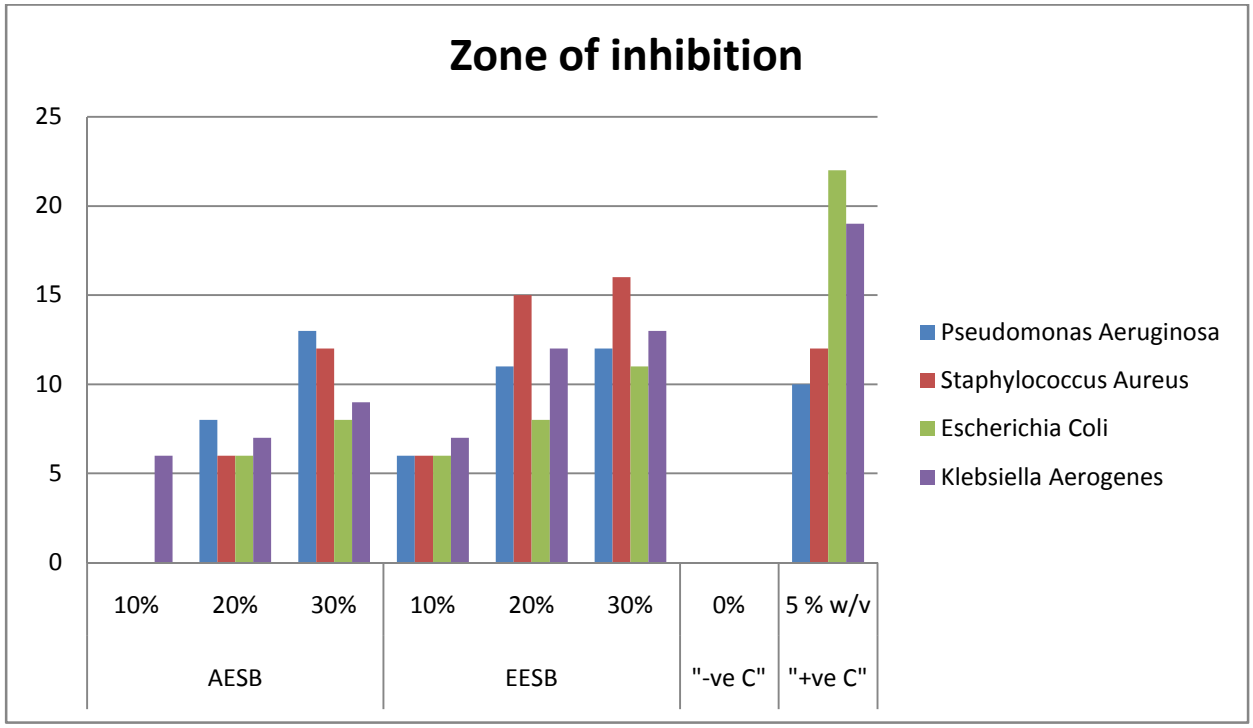


Graph 1.2: Showing Activity index of *Shigru* leaves against four bacterial strains

***Shigru* root bark:**

Bacteria	Zone of Inhibition (in mm)							
	AESB			EESB			-ve Control	+ve Control
	10 %	20 %	30 %	10 %	20 %	30 %	0 %	5 % w/v
Pseudomonas aeruginosa	0	8	13	6	11	12	0	10
Staphylococcus aureus	0	6	12	6	15	16	0	12
Escherichia coli	0	6	8	6	8	11	0	22
Klebsiella aerogenes	6	7	9	7	12	13	0	19

Table 1.3: Showing zone of inhibition of *Shigru* root bark against four bacterial strainswhere AESB is aqueous extract of *shigru* root bark, EESB is ethanolic extract of *shigru* root bark

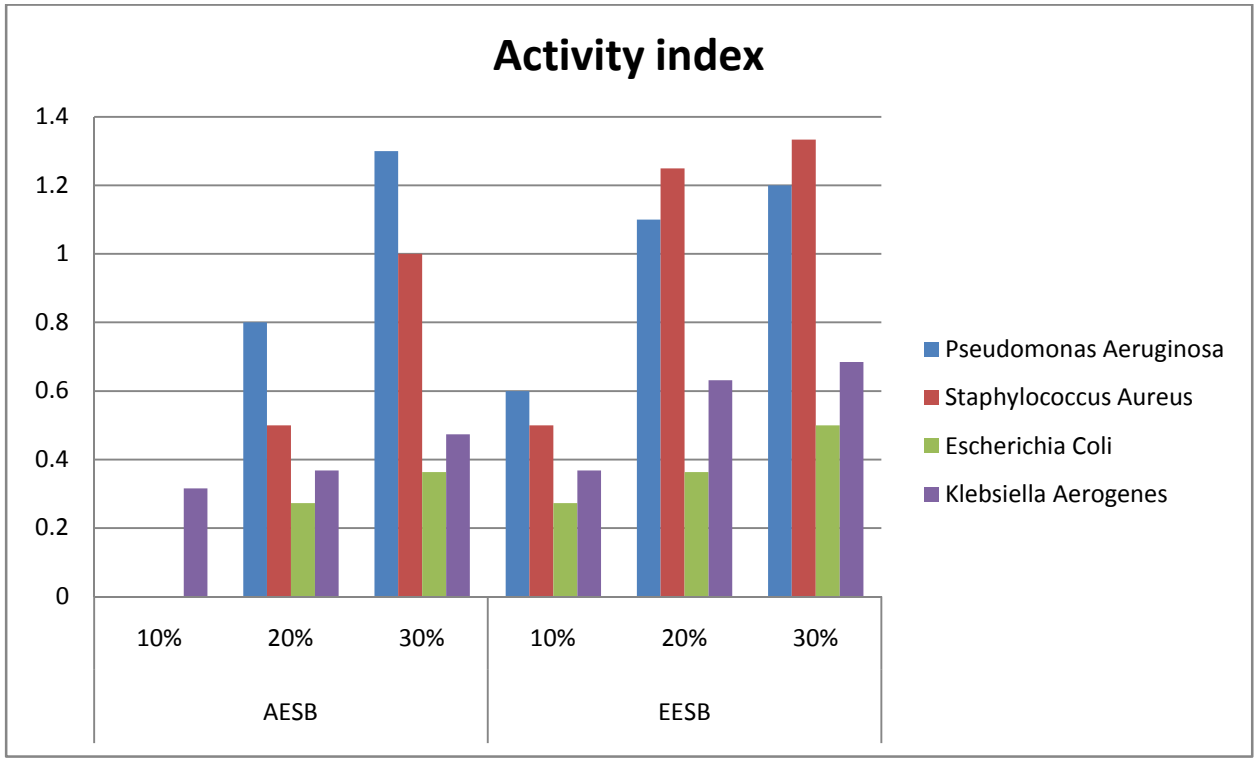


Graph 1.3: Showing zone of inhibition of *Shigru* root bark against four bacterial strains

Activity index:

Bacteria	Zone of Inhibition (in mm)					
	AESB			EESB		
	10%	20%	30%	10%	20%	30%
Pseudomonas aeruginosa	0	0.8	1.3	0.6	1.1	1.2
Staphylococcus aureus	0	0.5	1	0.5	1.25	1.3
Escherichia coli	0	0.27	0.36	0.27	0.36	0.5
Klebsiella aerogenes	0.31	0.36	0.47	0.37	0.63	0.68

Table 1.4: Showing activity index of *Shigru* root bark against four bacterial strains

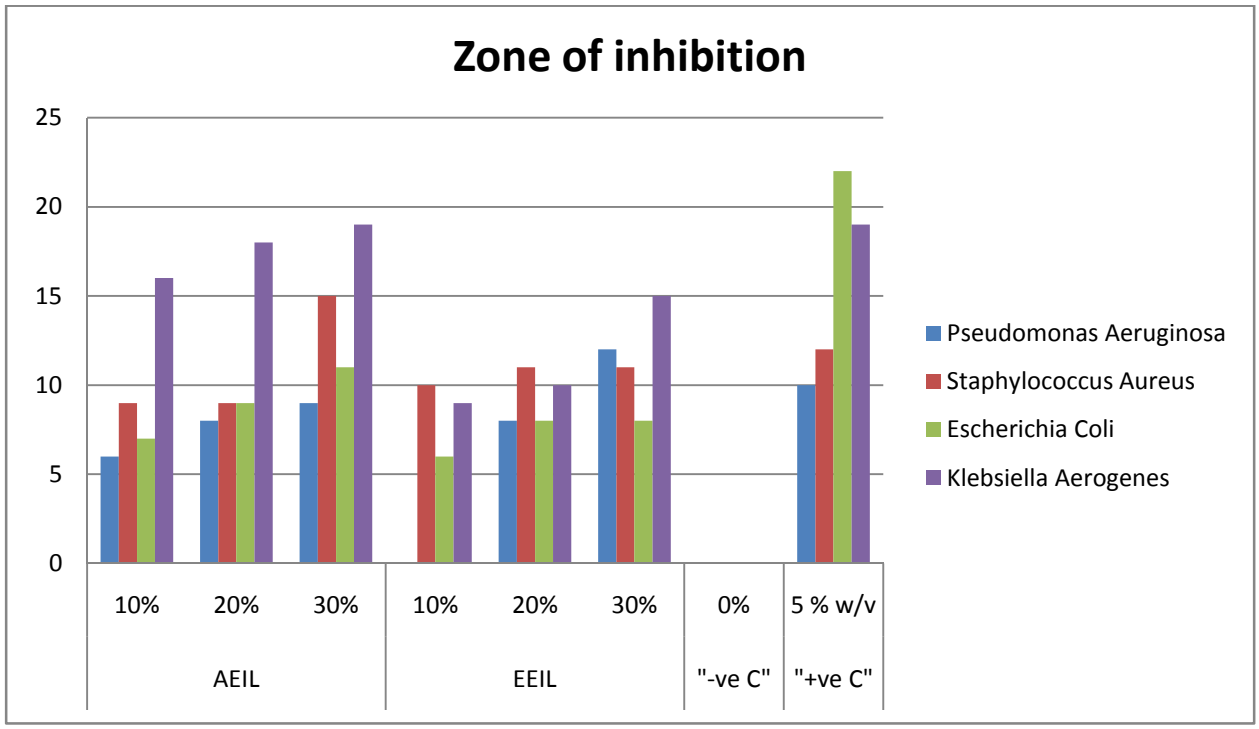


Graph 1.4: Showing activity index of *Shigru* root bark against four bacterial strains

***Ingudi* leaf:**

Bacteria	Zone of Inhibition (in mm)							
	AEIL			EEIL			-ve Control	+ve Control
	10 %	20 %	30 %	10 %	20 %	30 %	0 %	5 % w/v
Pseudomonas aeruginosa	6	8	9	0	8	12	0	10
Staphylococcus aureus	9	9	15	10	11	11	0	12
Escherichia coli	7	9	11	6	8	8	0	22
Klebsiella aerogenes	16	18	19	9	10	15	0	19

Table 1.5: Showing zone of inhibition of *ingudi* leaves against four bacterial strainswhere AEIL is aqueous extract of *ingudi* leaves, EEIL is ethanolic extract of *ingudi* leaves

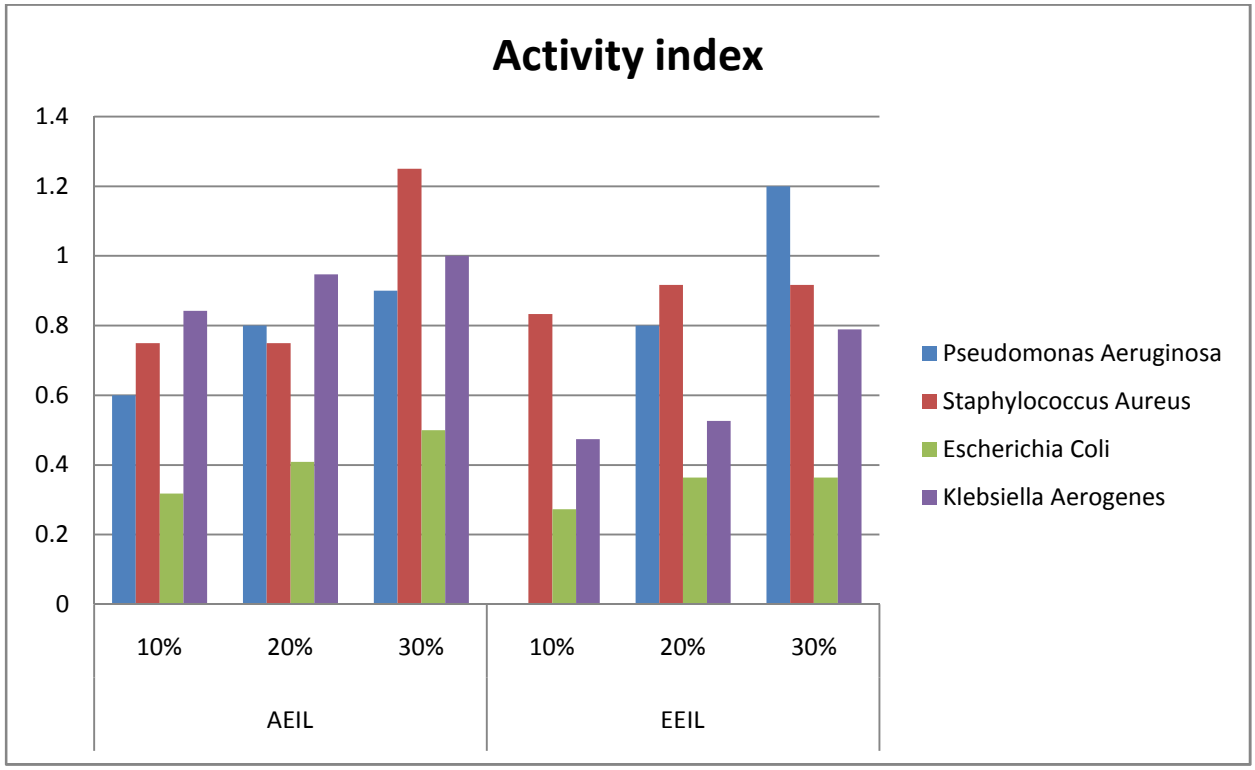


Graph 1.5: Showing zone of inhibition of *ingudi* leaves against four bacterial strains

Activity index:

Bacteria	Zone of Inhibition (in mm)					
	AEIL			EEIL		
	10%	20%	30%	10%	20%	30%
Pseudomonas aeruginosa	0.6	0.8	0.9	0	0.8	1.2
Staphylococcus aureus	0.75	0.75	1.25	0.83	0.92	0.92
Escherichia coli	0.32	0.41	0.5	0.27	0.36	0.36
Klebsiella aerogenes	0.84	0.95	1	0.47	0.53	0.79

Table 1.6: Showing activity index of *ingudi* leaves against four bacterial strains



Graph 1.6: Showing activity index of *ingudi* leaves against four bacterial strains

Mixed Solutions:

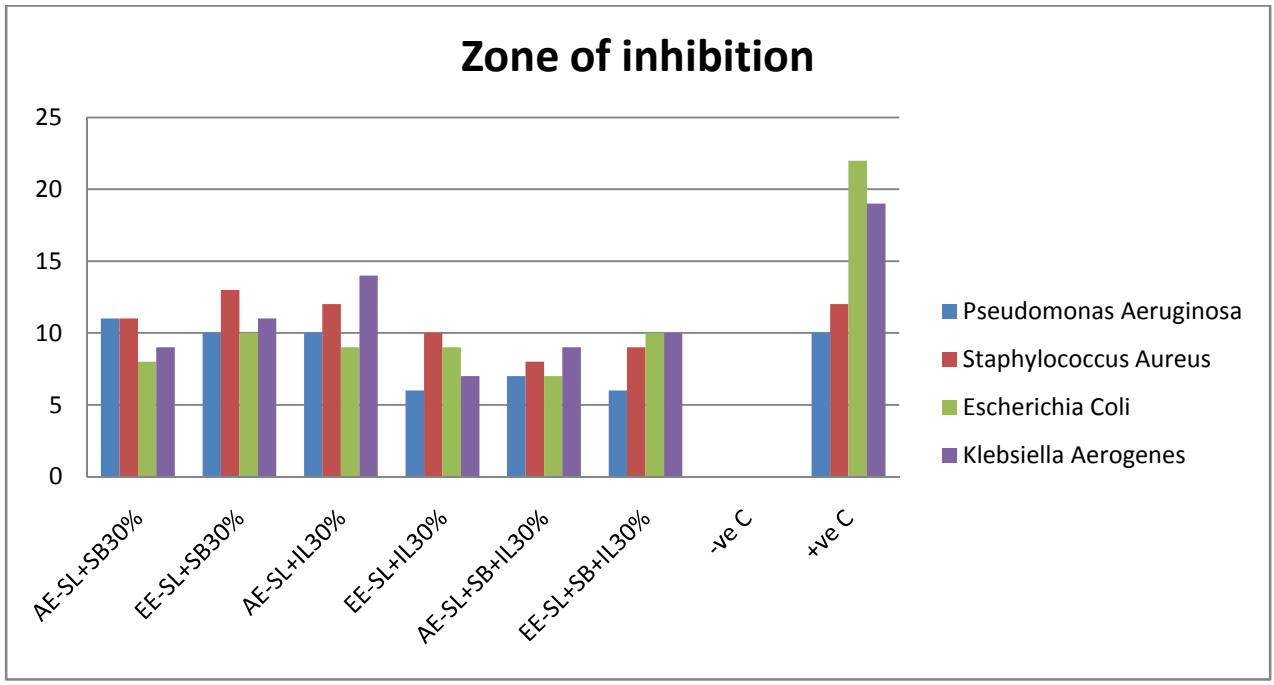
***Shigru* leaf + *Shigru* root bark- SL+SB**

***Shigru* leaf + *Ingudi* leaf- SL+IL**

***Shigru* leaf + *Shigru* root bark+ *Ingudi* leaf - SL+SB+IL**

Bacteria	Zone of Inhibition (in mm)							
	AE-SL+SB 30%	EE-SL+SB 30%	AE-SL+IL 30%	EE-SL+IL 30%	AE-SL+SB+IL 30%	EE-SL+SB+IL 30%	-ve C	+ve C
Pseudomonas aeruginosa	11	10	10	6	7	6	0	10
Staphylococcus aureus	11	13	12	10	8	9	0	12
Escherichia coli	8	10	9	9	7	10	0	22
Klebsiella aerogenes	9	11	14	7	9	10	0	19

Table 1.7: Showing zone of inhibition of mixtures of test samples against four bacterial strains

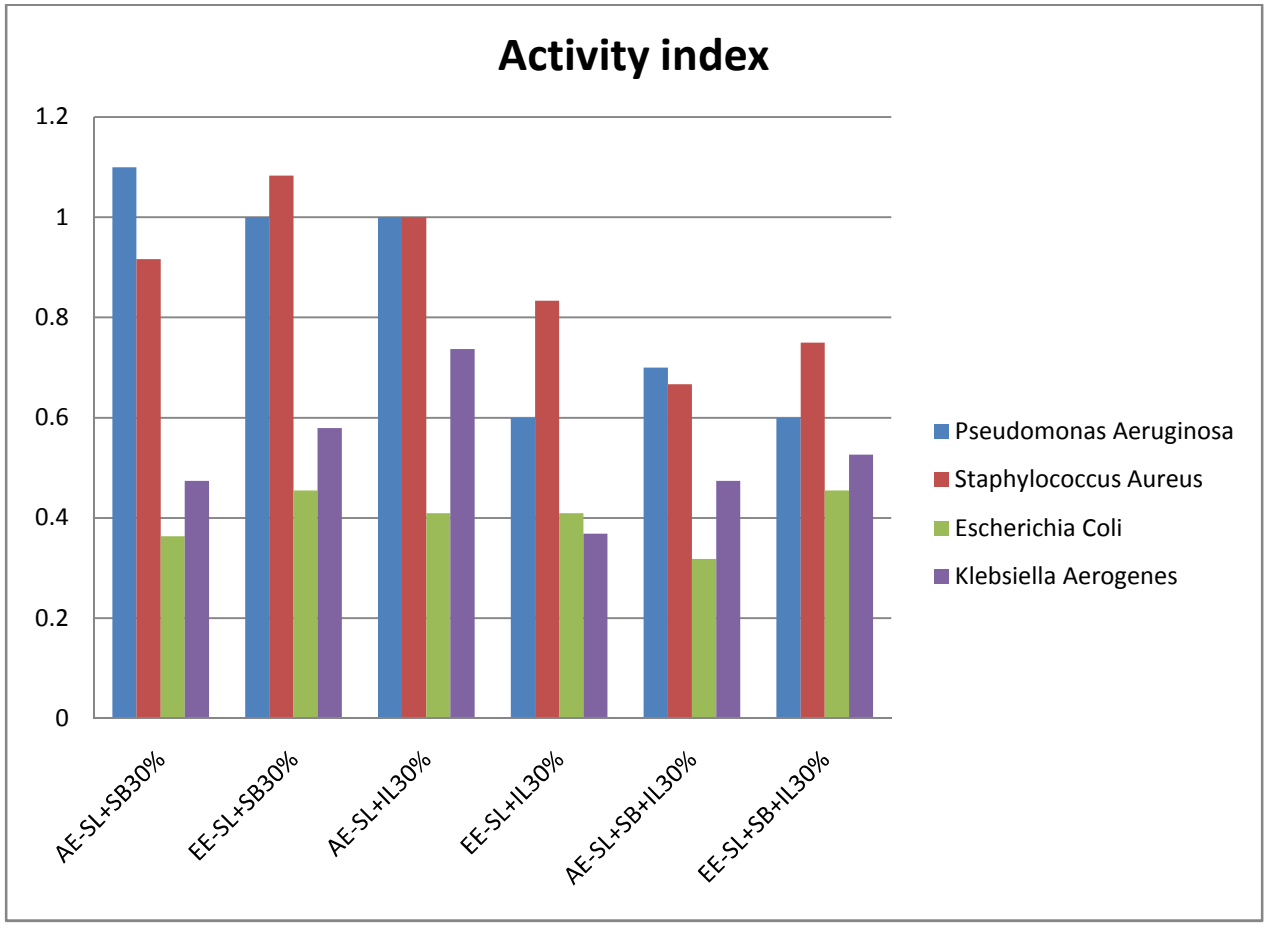


Graph 1.7: Showing zone of inhibition of mixtures of test samples against four bacterial strains

Activity index:

Bacteria	Zone of Inhibition (in mm)					
	AE-SL+SB 30%	EE-SL+SB 30%	AE-SL+IL 30%	EE-SL+IL 30%	AE-SL+SB+IL 30%	EE-SL+SB+IL 30%
Pseudomonas aeruginosa	1.1	1	1	0.6	0.7	0.6
Staphylococcus aureus	0.92	1.08	1	0.83	0.66	0.75
Escherichia coli	0.36	0.45	0.41	0.41	0.32	0.45
Klebsiella aerogenes	0.47	0.58	0.74	0.37	0.47	0.56

Table 1.8: Showing activity indexes of mixtures of test samples against four bacterial strains



Graph 1.8: Showing activity index of mixtures of test samples against four bacterial strains

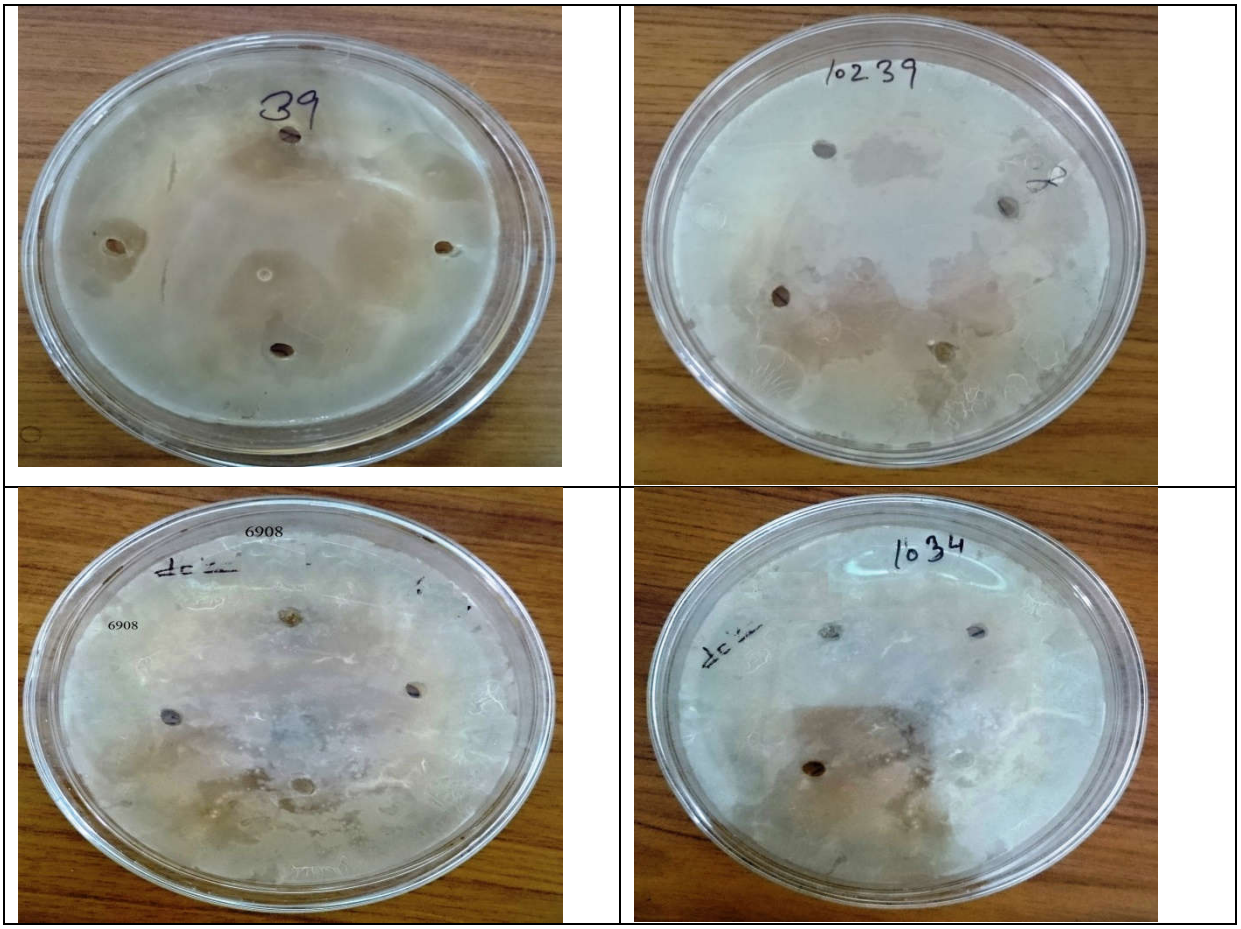
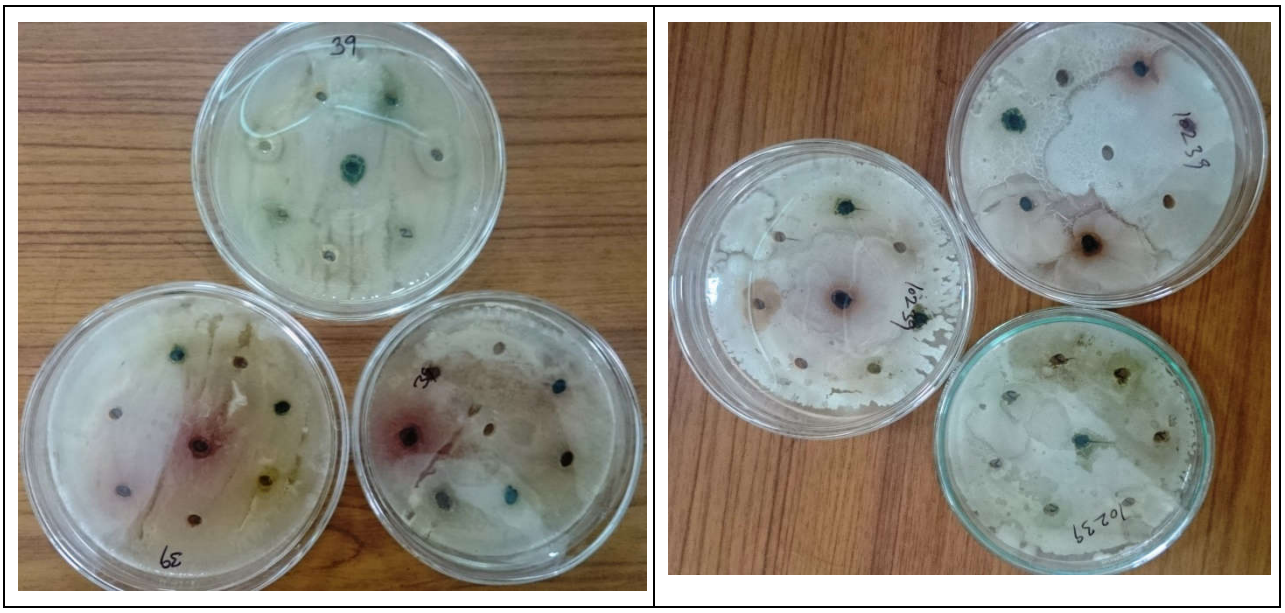


Fig 1.4: Zone of inhibition of + ve & -ve control against four strains of bacteria



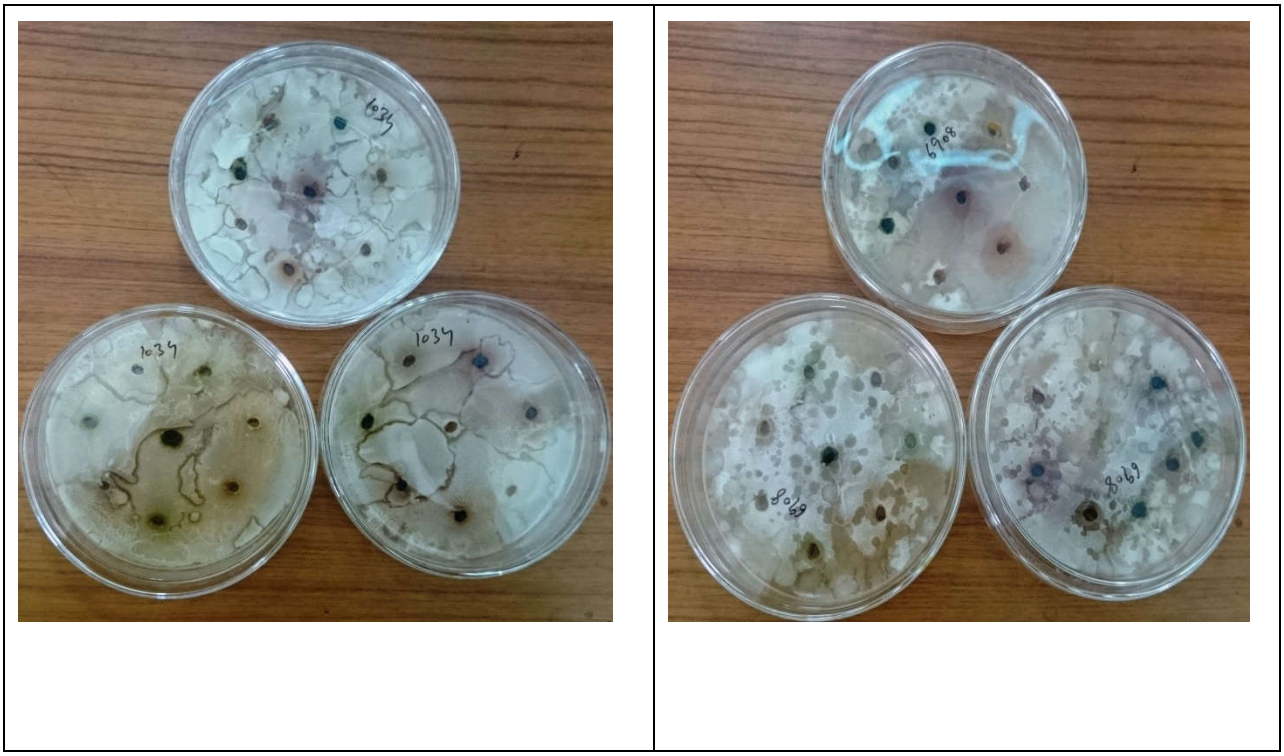


Fig 1.5: Showing zone of inhibition of test groups against four strains of bacteria

DISCUSSION:

The bioassay results for antimicrobial activity of the Aqueous and Ethanol extracts of all samples are presented in Tables. From the results it is very clear that both ethanol and Aqueous extracts of all samples inhibited the growth of all the tested strains of bacteria.

But best results were shown by aqueous extract of *Ingudi* leaf with zone of inhibition of 15mm, 11mm & 19mm against Staphylococcus aureus, *Escherichia Coli &Klebsiella aerogenes* respectively at 30% concentration with activity index of 1.25, 0.5 & 1 respectively. It showed potent antimicrobial activity against*Pseudomonas aeruginosa*with activity index of 0.9

Mixtures of aqueous extracts of *shigru* leaf, *shigru* root bark &*ingudi* leaf does not show good results. But their ethanolic extract showed some results on *Escherichia Coli,Klebsiella aerogenes* with zone of inhibition of 10mm.

With respect to activity index, Mixed ethanolic extracts of *Shigru* leaf, *ingudi* leaf &*shigru* bark is effective against *Pseudomonas aeruginosa, Staphylococcus aureus &Klebsiella aerogenes* with activity index of 0.6, 0.75 & 0.52 respectively.

CONCLUSION:

From the results, it is very clear that individual drugs showed good effects on all four bacterial strains.Ethanolic and aqueous extract showdissimilar inhibitory response due to difference in plant metabolites.

After mixing of ethanolic extracts and aquous extracts of *shigru* leaf, *shigru* root bark &*ingudi* leaf, inhibitory response go downwards. It may be possible that test samples have antagonistic effects or low concentration of active ingredients made inhibitory response to go downwards.

This study infers that decoction or paste of individual drugs can be used for washing or local application on surgical wounds but mixture should be avoided as it will not give satisfactory results.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED