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## A COMPARATIVE ANTIMICROBIAL EVALUATION OF *EMBELIA RIBES* BURM. F. AND *EMBELIARO BUSTA* AUCT. NONROXB. FRUITS

Malvika Saini\*, Gaurav Sharma and Mita Kotecha

Department of Dravyagunavigyan, National Institute of Ayurveda, Jaipur, Rajasthan, India.

### Keywords:

*Embelia ribes burm. f.*,  
*Embeliario busta auct. Nonroxb.*,  
Bacteria, Zone of inhibition,  
Activity index, IC<sub>50</sub> value

### Correspondence to Author:

**Dr. Malvika Saini**

P.G. Scholar

Department of Dravyagunavigyan,  
National Institute of Ayurveda,  
Jaipur, Rajasthan, India.

**E-mail:** malvikasaini.ms@gmail.com


**ABSTRACT: Background:** *Vidang (Embelia ribes Burm. f.)* is a very famous plant for its anthelmintic activity. In Ayurveda, it is a first choice of drug for its *krimighna* action or to kill foreign pathogens. *Embeliario busta auct. Nonroxb.*, the other species of *vidang* is adulterated in *Embelia ribes Burm. f.* Therefore *shastrokta* or original *vidang* and its adulterant species were selected for the comparative study. **Methods:** Both aqueous and alcoholic extracts of fruits of both species of *Embelia* at 10, 20, 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 were determined, then activity index and IC<sub>50</sub> value was calculated. **Results:** Both ethanol and Aqueous extracts of *Embelia ribes Burm. f.* and *Embeliario busta auct. Nonroxb.* inhibited the growth of all the tested strains of bacteria. Aqueous extract *Embelia ribes Burm. f.* has more potent action against microorganism *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, ZOI (16mm, 14mm, 19mm respectively), AI (0.64, 0.78, 0.70 resp.), IC<sub>50</sub> (1.751, 1.166, 1.641 respectively). **Conclusion:** *Embelia ribes Burm. f.* has more potent action in comparison to *Embeliario busta auct. Nonroxb.*. It concludes *shastrokta vidang* species (*Embelia ribes Burm. f.*) is better to its adulterant species (*Embeliario busta auct. Nonroxb.*).

**INTRODUCTION:** *Embelia ribes* Burm. f. is a threatened woody shrub belongs to the family Myrsinaceae, which is found throughout India up to an altitude of 1600 m, from Central Himalaya to Konkan, Deccan, Western Ghats and South India. In 'Ayurveda', the plant is popularly known as *Vidanga* or *Bashmak* or *Krimigna* (Sanskrit); *Baberangor Wawrung* (Hindi); *Vayuvilanga* (Kannada) and it is used as one of the adjuvant in most of the drug preparations. The fruits contain embelin, quercitol, tannin, christembin, embolic acid, fatty ingredients, resinoid, volatile oil, vilangin.

The plant possess nematicidal, estrogenic, hypoglycaemic, anthelmintic, antibiotic, anti-tubercular, anti-implantation, anti-ovulatory, antifertility, anti-inflammatory, hypotensive, antipyretic, diuretic, hepato protective, anti-leishmanial, resorptive, anti-spermatogenic, anti-androgenic, anticancer, immunostimulant property.

Fruits are astringent, bitter useful in helminthiasis, skin diseases, leprosy, pruritis, nervous debility, amentia, dyspepsia, jaundice, flatulence, colic, constipation, strangury, tumour, asthma, bronchitis, dental caries, odontalgia, hemicrania, dyspnoea, cardiac diseases, psychological disorder, ringworm, fever, emaciation, general debility<sup>1</sup>.

Fruits of *Embeliario busta auct. Nonroxb.* are used as adulterant to *Embelia ribes* Burm. f.. At some places it is used as substitute of *E. ribes*. These seeds are found in abundance and *E. ribes* now a day is considered to be in the red listed plants.

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*E.robusta* has similar properties to *E. ribes*. and chemical constituents like embelin etc. In Market, in the name of *Vidang*, both species are sold mostly in mixed form. At some places only *Embeliaro busta* auct. Nonroxb. is available in the name of *Vidang*. It is a rambling shrub or small tree, distributed from Ceylon to Malabar coast through Sylhet and Assam to Singapore <sup>2</sup>. Both the two species are different in colour and size, *E. ribes* is black in colour, small and rounded whereas *Embeliaro busta* auct. Nonroxb. auct. is reddish brown in colour, big in size as compared to *E. ribes*

and rounded. Both possess same actions according to modern literature. But researches on both species have not been done so far. So both species were selected for the comparative study. Moreover fruit of *vidang* is one of the ingredient of a paste formulation mentioned for local application in wound infections like fistula in ano, piles etc. <sup>3</sup>, So to prove this activity of *Vidang* experimentally, *in vitro* study was carried out with both species of *Vidang* and following wound infection causative organisms.



**FIG. 1: SHOWING FRUITS OF *EMBELIA RIBES* BURM. F.**



**FIG. 2: SHOWING FRUITS OF *EMBELIARO BUSTA* AUCT. NONROXB.**

**MATERIAL AND METHODS:**

**Plant material:** Fruits of *Embelia ribes* Burm. f. were collected from Ayurveda Regional Research Institute, Itanagar and were authenticated by the same i.e. Ayurveda Regional Research Institute, Itanagar with Authentication no. – 2655. Fruits of *Embeliario busta* auct. Nonroxb. were collected from Joginder Nagar, Himachal Pradesh and authentication was done at herbarium section, Department of Botany, Rajasthan University, Jaipur with authentication no. RUBL211596.

**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 and 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 °C, to constant weight and weighed<sup>4</sup>. The yield was 6.3% for ethanol extract of *Embelia ribes* Burm. f., 1.31% for aqueous extract of *Embelia ribes* Burm. f., 11.45% for ethanol extract of *Embeliario busta* auct. Non roxb., 2.94% for aqueous extract of *Embeliario busta* auct. Nonroxb.. Repeated the procedure for 5 times so as to collect the enough amount of extract required for antimicrobial activity. For carrying out antimicrobial activity, 10%, 20%, 30% concentrations of both ethanol and 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*, *Klebsiellaaerogenes*. Out of which *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiellaaerogenes* 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 and 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 and 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. Like all other living forms, micro-organisms need suitable nutrients and favorable environments for growth. A simple way to obtain bacteria is to grow them in a flask in broth medium.

100 ml Nutrient broth medium were transferred in conical flasks (of quantity 100ml) 20ml each. The flasks were capped with cotton plug and autoclaved at 121 °C for 20 minutes at 15 lb pressure per square inch. Dried and freezed bacteria were transferred to conical flasks with nutrient broth media, kept at 37 °C to get cultures.

**Preparation of media and media plates:** Muller-hinton 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 °C 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 37 °C 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 37 °C 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<sup>5</sup>, because of reproducibility and precision. Wells (of about 4 mm diameter) were made on the plates with the

help of sterile stainless steel borer. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates. About 20-30 $\mu$ l different concentrations of plant solvent extracts were added using sterile syringe into the wells and allowed to diffuse at room temperature for 2hrs. 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 fruits of *Embelia ribes* Burm. f..

10%, 20%, 30% concentrations of aqueous extracts of fruits of *Embelia ribes* Burm. f..

10%, 20%, 30% concentrations of ethanol extracts of fruits of *Embeliario busta* auct. Non roxb..

10%, 20%, 30% concentrations of aqueous extracts of fruits of *Embeliario busta* auct. Non roxb..

**Standard group-** 5% w/v Vancomycin

**Negative control group-** Distilled water and Ethanol

**Determination of activity index:** The activity index of the crude plant extract was calculated as <sup>6</sup>:

### RESULTS:

#### Zone of Inhibition in mm:

**TABLE 1: SHOWING ZONE OF INHIBITION OF TEST DRUGS AND POSITIVE, NEGATIVE CONTROL GROUPS**

	AEERib			EEERib			AEERob			EEERob			-ve Control	+ve Control
	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %	10 %	20 %	30 %	0 %	5 % w/v
<i>Pseudomonas aeruginosa</i>	7	11	18	9	10	12	9	11	11	12	14	17	0	20
<i>Staphylococcus aureus</i>	6	9	16	8	9	11	0	0	13	6	7	10	0	25
<i>Escherichia coli</i>	10	11	14	9	10	13	0	6	12	7	8	10	0	18
<i>Klebsiella aerogenes</i>	8	11	19	9	11	14	0	7	12	5	6	8	0	27

Where AEERib- aqueous extract of *Embelia ribes* Burm.f., EEERib- ethanol extract of *Embelia ribes* Burm.f., AEERob- aqueous extract of *Embeliario busta* auct. Non roxb., EEERob- ethanol extract of *Embeliario busta* auct. Non roxb. at 10%, 20%, 30% concentrations

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

**IC<sub>50</sub>: The half maximal inhibitory concentration (IC<sub>50</sub>)** is a measure of the effectiveness of a substance in inhibiting a specific biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is needed to inhibit a given biological process (or component of a process, i.e. an enzyme, cell, cell receptor or microorganism) by half. The IC<sub>50</sub> of a drug can be determined by constructing a dose-response curve and examining the effect of different concentrations of antagonist on reversing agonist activity. IC<sub>50</sub> values can be calculated for a given antagonist by determining the concentration needed to inhibit half of the maximum biological response of the agonist. IC<sub>50</sub> values can be used to compare the potency of two antagonists <sup>7</sup>. In general, the higher the concentration of inhibitor, the more agonist activity will be lowered. IC<sub>50</sub> value increases as agonist concentration increases.

Determination of IC<sub>50</sub> <sup>8</sup>

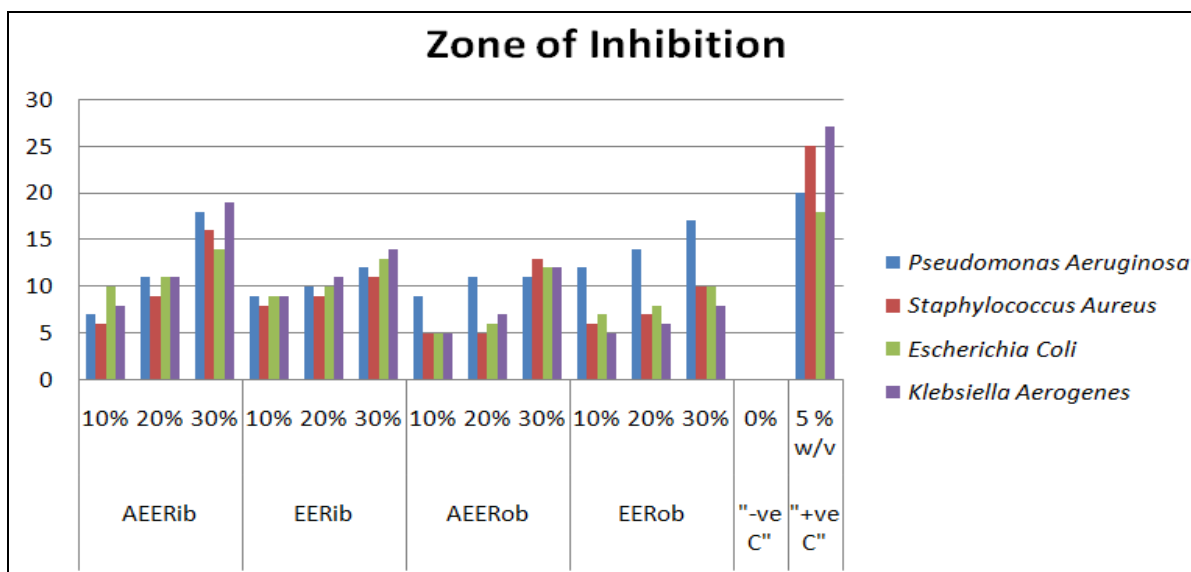
y-axis=% inhibition and x-axis= log conc.

linear equation y=mx + c.

Y= 50, x=log conc.

IC<sub>50</sub>- Antilog of X





GRAPH 1: SHOWING ZONE OF INHIBITION OF TEST SAMPLES WITH POSITIVE AND NEGATIVE CONTROLS WHERE C" STANDS FOR CONTROL GROUP

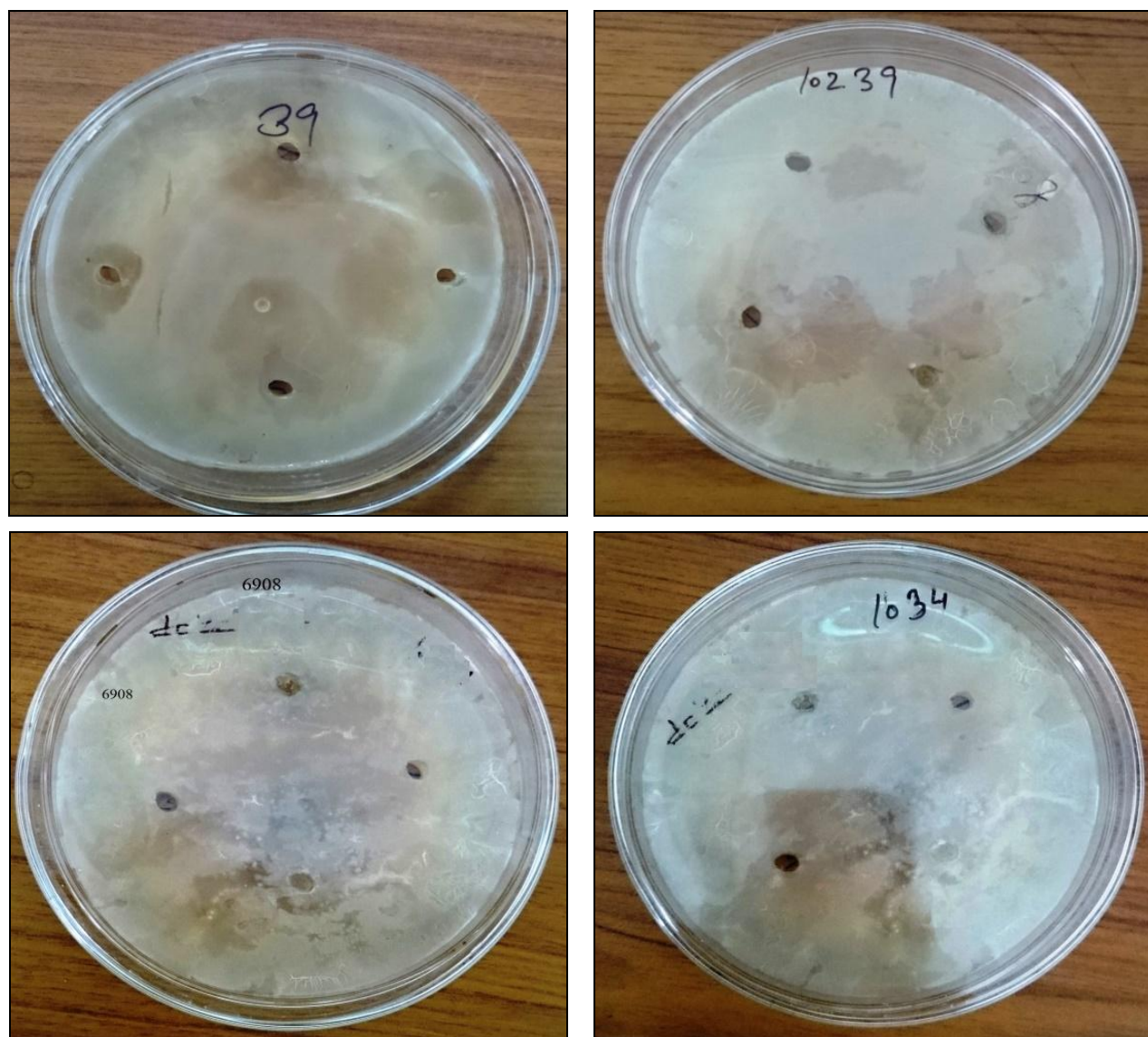


FIG. 3: ZONE OF INHIBITION OF + VE AND -VE CONTROL AGAINST FOUR STRAINS OF BACTERIA

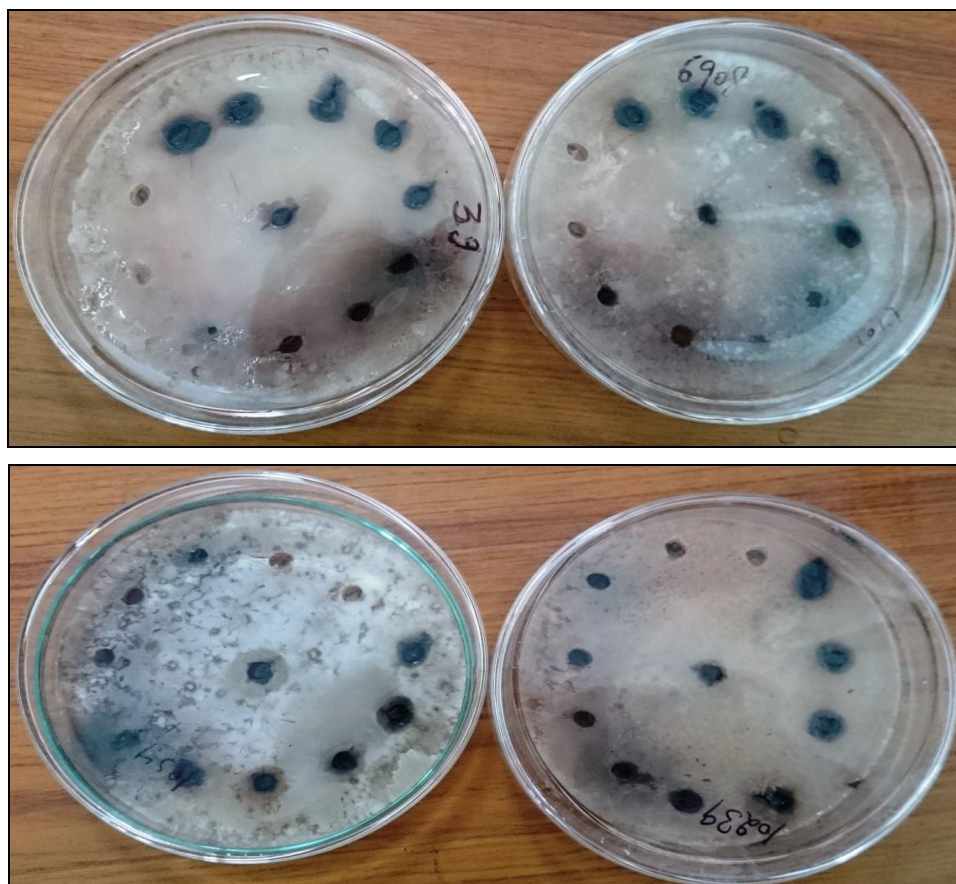
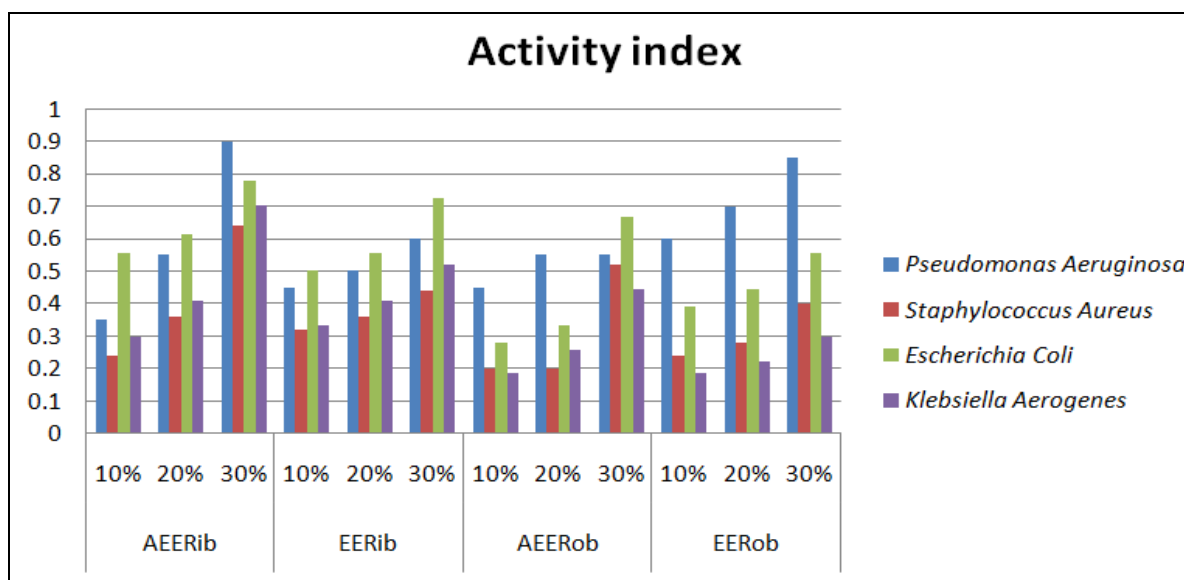


FIG. 4: ZONE OF INHIBITION OF TEST GROUPS AGAINST FOUR DIFFERENT STRAINS OF BACTERIA

Activity index:

TABLE 2: SHOWING ACTIVITY INDEX OF TEST DRUGS

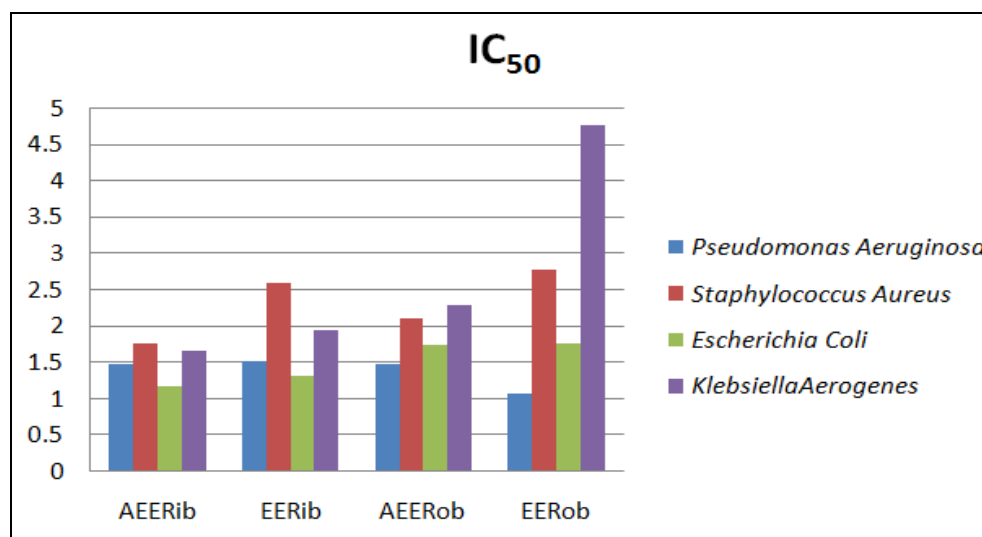
	AEERib			EERib			AEERob			EERob		
	10%	20%	30%	10%	20%	30%	10%	20%	30%	10%	20%	30%
<i>Pseudomonas aeruginosa</i>	0.35	0.55	0.9	0.45	0.5	0.6	0.45	0.55	0.55	0.6	0.7	0.85
<i>Staphylococcus aureus</i>	0.24	0.36	0.64	0.32	0.36	0.44	0.2	0.2	0.52	0.24	0.28	0.4
<i>Escherichia coli</i>	0.56	0.61	0.78	0.50	0.56	0.72	0.28	0.33	0.67	0.39	0.44	0.56
<i>Klebsiella aerogenes</i>	0.30	0.41	0.70	0.33	0.41	0.52	0.19	0.26	0.44	0.19	0.22	0.30



GRAPH 2: SHOWING ACTIVITY INDEXES OF TEST DRUGS

**IC<sub>50</sub> value:****TABLE 3: SHOWING IC<sub>50</sub> VALUE OF TEST DRUGS**

	AEERib	EERib	AEERob	EERob
<i>Pseudomonas aeruginosa</i>	1.457	1.506	1.468	1.063
<i>Staphylococcus aureus</i>	1.751	2.577	2.093	2.765
<i>Escherichia coli</i>	1.166	1.308	1.730	1.756
<i>Klebsiella aerogenes</i>	1.641	1.935	2.276	4.761

**GRAPH 3: SHOWING IC<sub>50</sub> VALUE OF TEST DRUGS**

**DISCUSSION:** The bioassay results for antimicrobial activity of the Aqueous and Ethanol extracts of *Embelia ribes* Burm. f. and *Embeliario busta* auct. Nonroxb. are presented in **Table 1**. From the results it is very clear that both ethanol and Aqueous extracts of *Embelia ribes* Burm. f. and *Embeliario busta* auct. Nonroxb. inhibited the growth of all the tested strains of bacteria.

The Aqueous extract *Embelia ribes* Burm. f. was found privileged Zone of Inhibition against *S. aureus*, *E. coli*, *K. aerogenes* in comparison of ethanolic extract *Embelia ribes* Burm. f., Aqueous *Embeliario busta* auct. Nonroxb. extract, ethanolic *Embeliario busta* auct. Nonroxb. extract. But ethanolic extract of *Embeliario busta* auct. Non roxb. shows significant results on *P. aeruginosa* in comparison to ethanolic extract of *Embelia ribes* Burm. f. and aqueous *Embeliario busta* auct. Non roxb. extract. The activity index of the test substance above 0.5 is considered as significant activity<sup>9</sup>. Aqueous extract of *Embelia ribes* Burm. f. has activity index of 0.9, 0.64, 0.78, 0.70 against *P. aeruginosa*, *S. aureus*, *E. coli*, *K. aerogenes* respectively which again proves its better efficacy than other tests samples i.e. ethanolic extracts of

*Embelia ribes* Burm. f., aqueous extracts and ethanolic extracts of *Embeliario busta* auct. Nonroxb.. Ethanolic extract of *Embeliario busta* auct. Non roxb. Shows activity index of 0.85 against *Pseudomonas aeruginosa* which shows its potent action in comparison to other samples. Concentration required to inhibit 50 % of *Pseudomonas aeruginosa* is lowest in ethanolic extract of *Embeliario busta* auct. Nonroxb. (IC<sub>50</sub>=1.063) which depicts its more potent action as compare to other Extracts of *Embelia ribes* Burm. f. and *Embeliario busta* auct. Nonroxb.. Against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes*, IC<sub>50</sub> is lowest in aqueous extract of *Embelia ribes* Burm. f. i.e. 1.751, 1.166, 1.641 respectively required to kill 50% microorganism.

**CONCLUSION:** The difference in the activity may be due to the different secondary metabolites present in the ethanol and water extracts. Different solvents have various degrees of solubility for different phytoconstituents<sup>10</sup>. This indicates that the secondary metabolites act as an antimicrobial compounds, which either inhibit or kill the bacteria by different mechanisms.



Aqueous extract of *Embelia ribes* Burm. f. shows potent anti-microbial action against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella aerogenes* microorganism in comparison of ethanolic extract of *Embelia ribes*, aqueous extract of *Embeliario busta*, ethanolic extract of *Embeliario busta* auct. Nonroxb.. Ethanolic extract of *Embeliario busta* auct. Nonroxb. shows potent action against *Pseudomonas aeruginosa*, microorganism.

Out of four tested microorganisms, three organisms are affected by aqueous extract of *Embelia ribes* Burm. f. upto a significant level which infers that *Embelia ribes* Burm. f. has more potent action in comparison to *Embeliario busta* auct. Non roxb.. It represents *shastroktavidang* species (*Embelia ribes* Burm. f.) is better than its adulterant species (*Embeliario busta* auct. Nonroxb.).

#### CONFLICTS OF INTEREST STATEMENT:

There are no conflicts of interest.

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