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# ASSESSMENT OF ANTIBACTERIAL EFFECT IN *ALAMBUSHA* (*BIOPHYTUM SENSITIVUM* LINN.) LEAF AND ROOT EXTRACTS: A COMPARATIVE *IN VITRO* STUDY

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**ABSTRACT**

The plant selected in the present study for analysing the antibacterial activity is, Alambusha *(Biophytum sensitivum)*. The whole part of the plant is considered useful and exhibits krimighna, vrana ropana and pitta kaphaghna action as per Ayurveda. Also, it has been said by the folklore people that the leaves exhibit styptic action. So combining all these views, the study goes step by step to compare the Antibacterial activity of the leaf, root and leaf root combination of Alambusha in three different extraction solvents (Ethanol, chloroform, aqueous), which is taken at a fixed concentration of 20 mg/ml (2%) in an increasing volume of 25 ul, 50 ul, 75 ul, 100 ul against two wound infection-causing bacteria's such as *Pseudomonas aeruginosa* and *Bacillus subtilis,* by Agar well diffusion method. The study fortifies that leaf ethanolic extract of Alambusha at a volume of 100 microliters shows maximum antibacterial activity against both bacterial species, that too highest in *Pseudomonas aeruginosa* when compared to root alone and leaf along with root combinations. These results obtained show that plant extracts can be used as anti-infective agents.

**Keywords:** Krimighna, Alambusha, Bacteria

## INTRODUCTION

Natural antibiotics are natural products which can kill microorganisms and give health and hygiene. Ayurvedic science uses natural antibiotics to overcome the alarming occurrence of infectious diseases. Naturally, Ayurveda, the oldest healthcare system in the world, does not have the word "Antibiotics". [1] But searching deep into its literature shows several references stating that certain diseases are produced due to microorganisms (krimi). Acharya mentioned many drugs and kalpanas (Medicinal formulations) to kill these microorganisms. Nowadays, the most challenging clinical problem faced by clinicians is to heal wounds correctly without getting any kind of bacterial infections. [2] Wound, known by the term 'Vrana' in Ayurveda, refers to the Injury of the skin. Ayurveda is a unique science with many natural resources, having more extraordinary ability towards wound healing. Thus, the problem can be solved, by adopting a correct and efficient selection of a natural herb with good antibacterial activity, as mentioned in the Samhita.

This is an experimental study (*In-vitro*) in which the krimighna action of the plant parts of Alambusha (*Biophytum sensitivum*)[3] against two wound infections causing pathogenic bacteria was assessed. Even though the whole plant is useful, a different approach was taken for studying the antibacterial effect. Apart from selecting the leaf and root sample alone, a combination of leaf and root in an equal ratio was also compared with the individual samples to find the antibacterial effect. This is done to assess how the combined action of two different parts of the same plant works and to determine whether the combination will show more / less / no activity compared to the individual parts. Therefore, with this research, a small attempt has been made to scientifically prove the krimighna action of the Alambusha, as per the classics, in a different way. At the same time, the availability of plant material also needs to be considered; since the plant is seasonal growing [seen during the rainy season only], there might be a lack of availability of the plant, and also uprooting the whole plant for the therapeutic purpose can lead to the destruction of the whole species. This comparative study has studied whether to rule out the combination approach or not and to accept the individual approach. Thus, the availability problem of the plant species can also be solved, and an eco-friendly plant microbicide can be developed.

## MATERIALS AND METHODS

### Collection Identification and Processing of Trial Drug

The trial drug Alambusha was collected from the regions of Tamil Nadu State in its fresh form. The plant species have been identified and authenticated by the Taxonomist of Botanical Survey of India, Jodhpur. The leaf and roots of the identified plant species were taken for the study. These were washed with tap water, dried under shade and powdered.

### Preparation of Concentration of Extractions

A fixed concentration of 20 mg/ml (2%) of the trial samples (leaf, root, leaf along with root) was prepared for the antibacterial study. For this, an amount of 20 mg from each of the ethanol, chloroform, and aqueous extracts of leaf, root and leaf + root was taken in 6 Eppendorf tubes separately. Then add 1 ml of 100%

DMSO to each of these tubes and shake well. Keep it undisturbed for a few hours to properly mix the solution.

**Pathogenic Strains Selected for Study**

*Pseudomonas aeruginosa* = Gram Negative
*Bacillus subtilis* = Gram Positive [4]

In freeze-dried form, these were collected from the "Institute of Microbial Technology" {IMTECH} Chandigarh. The stock cultural maintenance and the antibacterial study were done at "Seminal Applied Science Private Limited", Jaipur.

**Antibacterial Sensitivity Study**

*In - vitro* antibacterial activity of the test, samples were studied by Agar well diffusion method. Muller Hinton Agar media was used as the culture media for the preparation of nutrient broth, which is used for the preparation of media plates in antibacterial studies[5]. The test sample extracts were prepared at 20 mg/ml concentration in the Eppendorf's and taken to the LAF chamber. Five wells were prepared in the seeded agar plates using a gel puncture. The size of all the wells is approximately 6 mm[6]. Then, using a pipette from each Eppendorf containing the test samples, a quantity of 25 microliters, 50 microliters, 75 microliters, and 100 microliters were taken and introduced into the respective wells, with the readings marked. A Standard Antibiotic named (Ciprofloxacin) of about 40 microliters at 1 mg/ml concentration was introduced into the well in the middle of the plates. The plates were then incubated overnight at 37 $^0$C in an incubator. The diameter (in mm) of the clear Zone of Inhibition (ZOI) was determined in terms of zone sizes around each well using a measuring scale[7].

**Table 1: Ethanolic Extracts**

| Samples | Zone of Inhibition of Ethanolic extracts at 20 mg/ml (2%) taken in an increasing volume (microliters) | | | | Standard at 1 mg/ml taken in an amount of 40 microliters |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| Leaf Extract | 23 | 26 | 31 | 32 | 49 |
| Root Extract | 10 | 12 | 13 | 15 | 49 |
| Leaf + Root Extract | 7 | 8 | 9 | 10 | 49 |

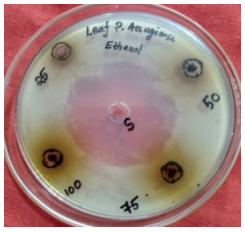


**Figure 1: Leaf Sample**

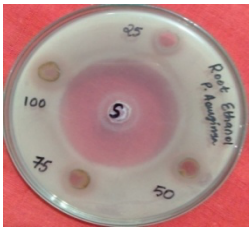


**Figure 2: Root Sample**

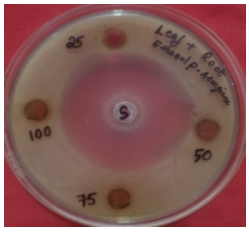


**Figure 3: Leaf + Root Sample**

**Table 2: Chloroform Extracts**

| Samples | Zone of Inhibition of Chloroform extracts at 20 mg/ml (2%) taken in an increasing volume (microliters) | | | | Standard at 1 mg/ml taken in an amount of 40 microliters |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| Leaf extract | 19 | 20 | 22 | 25 | 40 |
| Root extract | 8 | 9 | 10 | 11 | 40 |
| Leaf + Root extract | 3 | 3 | 2 | 1 | 40 |

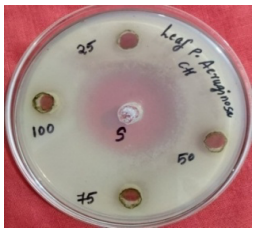


**Figure 4: Leaf Sample**

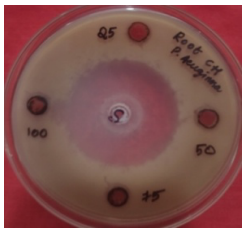


**Figure 5: Root Sample**

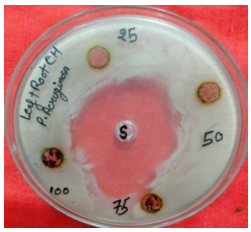


**Figure 6: Leaf + Root Sample**

**Table 3: Aqueous Extracts**

| Samples | Zone of Inhibition of Aqueous extracts at 20 mg/ml (2%) taken in an increasing volume (microliters) | | | | Standard at 1 mg/ml taken in an amount of 40 microliters |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| Leaf extract | 9 | 12 | 14 | 15 | 40 |
| Root extract | 10 | 11 | 12 | 14 | 40 |
| Leaf + Root extract | 9 | 12 | 13 | 14 | 40 |

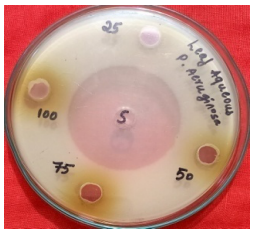

**Figure 7: Leaf Sample**

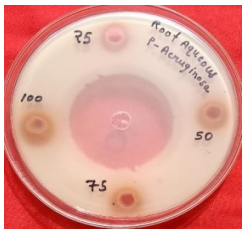

**Figure 8: Root Sample**

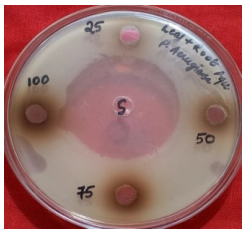

**Figure 9: Leaf + Root Sample**

**Table 4: Ethanolic extracts**

| Samples | Zone of Inhibition Ethanolic extracts at 20 mg/ml (2%) taken in an increasing volume (microliters) | | | | Standard at 1 mg/ml taken in an amount of 40 microliters |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| Leaf extract | 20 | 22 | 24 | 27 | 43 |
| Root extract | 10 | 11 | 13 | 15 | 43 |
| Leaf + Root extract | 3 | 7 | 10 | 10 | 43 |

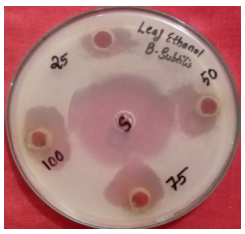

**Figure 10: Leaf Sample**

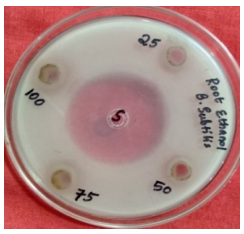

**Figure 11: Root Sample**

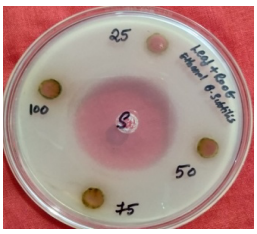

**Figure 12: Leaf + Root Sample**

**Table 5: Chloroform Extract**

| Samples | Zone of Inhibition of Chloroform extracts at 20 mg/ml taken in an increasing volume (microliters) | | | | Standard at 1 mg/ml taken in an amount of 40 microliters |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| Leaf extract | 20 | 21 | 21 | 23 | 40 |
| Root extract | 9 | 10 | 11 | 12 | 40 |
| Leaf + Root extract | 3 | 3 | 1 | 1 | 40 |

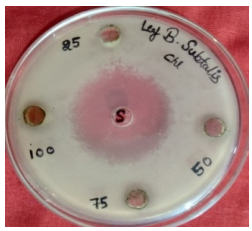

**Figure 13: Leaf Sample**

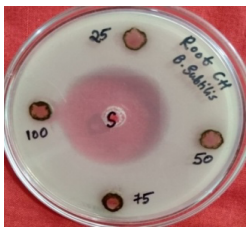

**Figure 14: Root Sample**

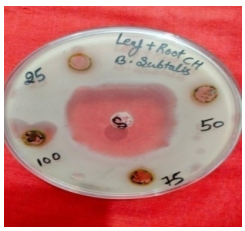

**Figure 15: Leaf + Root Sample**

**Table 6: Aqueous Extract**

| Samples | Zone of Inhibition Aqueous extracts at 20 mg/ml (2%) taken in an increasing volume (microliters) | | | | Standard at 1mg/ml taken in an amount of 40 microliters |
|---|---|---|---|---|---|
| | 25 | 50 | 75 | 100 | |
| Leaf extract | 9 | 10 | 15 | 16 | 40 |
| Root extract | 9 | 14 | 15 | 16 | 40 |
| Leaf + Root extract | 9 | 9 | 14 | 15 | 40 |

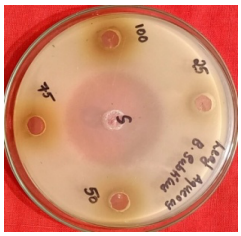

**Figure 16: Leaf Sample**

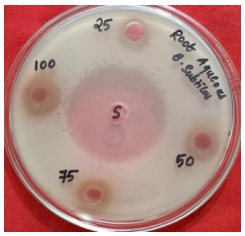

**Figure 17: Root Sample**

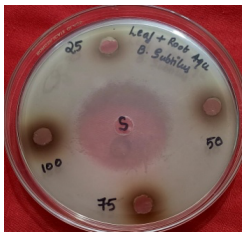

**Figure 18: Leaf + Root Sample**

## OBSERVATIONS AND RESULTS

The Relationship between Zone of Inhibition (ZOI) and drug sensitivity is determined based upon the scale developed by Arora D.S in 1997[8]. As per this scale, if ZOI is below 6, 6 to 9, 9 to 12, and above 12 are found to be insensitive, less sensitive, moderately sensitive and highly sensitive, respectively. The followings are the results and observations.

**Zone of Inhibition of each sample extract against *Pseudomonas aeruginosa***

**Ethanolic Extracts:** Among the ethanolic extracts of leaf, root and leaf+root of *Biophytum sensitivum,* the leaf ethanolic extract shows high sensitivity against *Pseudomonas aeruginosa* in all given volumes. The root ethanolic extract shows moderate sensitivity, and the combination of leaf and root ethanolic extract shows less sensitivity against *Pseudomonas aeruginosa.* Among all the volumes of leaf ethanolic extract, 100 microliters show a high sensitivity against *Pseudomonas aeruginosa*. This shows the dose dependency of the extract. (Table 1, Figure 1-3)

**Chloroform Extracts:** Among the chloroform extract of leaf, root, and leaf+root of *Biophytum sensitivum*, the leaf chloroform extract shows high sensitivity, the root chloroform extract shows a moderate sensitivity, whereas the combination of leaf and root chloroform extract was found to be insensitive against *Pseudomonas aeruginosa* in all given volume. Among all the volumes of leaf chloroform extract, 100 microliters are known to show a high sensitivity against *Pseudomonas aeruginosa.* (Table 2, Figure 4-6)

**Aqueous Extracts:** Here, the aqueous extract of all the samples [leaf, root, leaf+ root] shows moderate sensitivity towards *Pseudomonas aeruginosa*. And among the different extract volumes, 100 microliters show the maximum inhibition zone. (Table 3, Figure 7-9)

**Zone of Inhibition of each sample extract against *Bacillus subtilis***

**Ethanolic Extracts:** Among the ethanolic extract of leaf, root, and leaf+root of *Biophytum sensitivum*, the leaf ethanolic extract shows high sensitivity against *Bacillus subtilis* in all given volumes. Whereas root ethanolic extracts and the combination of leaf and root ethanolic extract show moderate sensitivity against *Bacillus subtilis*. Among all the volumes of leaf ethanolic extract, 100 microliters show a high sensitivity against *Bacillus subtilis*. (Table 4, Figure 10-12)

**Chloroform Extracts:** Among the chloroform extract of leaf, root, and leaf+root of *Biophytum sensitivum*, the leaf chloroform extract shows high sensitivity, and the root chloroform extract shows a moderate sensitivity against *Bacillus subtilis* in all given volumes. Whereas the combination of leaf and root chloroform extract is found to be insensitive against *Bacillus subtilis*. Among all the volumes of leaf chloroform extract, 100 microliters show a high sensitivity against *Bacillus subtilis*. (Table 5, Figure 13-15)

**Aqueous Extracts:** Here, 100 microliters of aqueous extract from all three samples show high sensitivity towards *Bacillus subtilis* bacteria. Whereas 25 microliters, 50 microliters, and 75 microliters of all three samples show moderate sensitivity towards *Bacillus subtilis*. (Table 6, Figure 16-18)

## DISCUSSION

The entire study is explained by comparing the antibacterial effect of 3 different samples of *Biophytum sensitivum*, {leaf powder, root powder, leaf along with root powder} in 3 different polar solvents [ethanol, chloroform, aqueous], against two different bacteria [*Pseudomonas aeruginosa* and *Bacillus subtilis*].

**Overall assessment of Antibacterial Activity**

The leaf ethanolic extract of Alambusha (*Biophytum sensitivum*) at a volume of 100 microlitre shows maximum antibacterial activity against both bacterial species, that too highest in *Pseudomonas aeruginosa* when compared to root and leaf along with root extractions.

**Comparison of Antibacterial Activity among the Bacterial Strains**

On analysing the antibacterial activity of the bacterial strains, it has been found that, in all the three extraction solvents of all three samples, *Pseudomonas aeruginosa* shows good sensitivity than *Bacillus subtilis.* Various pathways were used to explain the antimicrobial activity, but it is challenging to pinpoint the exact path due to the unavailability of sophisticated scientific techniques. There are many factors and circumstances which may affect the operation of antimicrobials like its concentration, number of species, presence of organic matter, temperature, and pH of the environment. [8] Considering Alambusha's vrana ropana action and through the above assessment, it has been found that the plant is therapeutically helpful in case of wound infections, caused due to *Pseudomonas aeruginosa* than *Bacillus subtilis*

**Comparison of Antibacterial Activity among the Plant Samples**

All the plant samples, leaf, root and leaf, and root combination show antimicrobial activity with varying results. Even then, among all the plant samples, the leaf sample of all three extracts shows maximum (ZOI) against both the strains selected for the study, the highest in *Pseudomonas aeruginosa*. A root sample against both bacteria follow this. And the combination sample exhibits the least sensitivity against both bacteria. The difference in antimicrobial activity is due to the difference in yield, component and concentration of active principle in these samples. The samples' extractive values also show the same range: the leaf sample's maximum percentage and the combined sample's minimum percentage. Minimum activity in combination samples might be due to forming of any new molecular compound in the sample extractives, which may inhibit the antimicrobial ability. Maximum activity in the leaf sample is because it has a high concentration of active principles in its sample extractives than the other samples. [9] The classical view of the krimighna action of Alambusha is thus proved. Also, through this research, it has been understood that the combination approach shows less activity, even though the combination's individual component shows antibacterial activity. Thus, the leaves alone of Alambusha can be used for developing a plant microbicide that too eco-friendly since there is no need for taking the whole plant.

**Comparison of Antibacterial Activity among the Plant Extracts**

Among all three extracts, the ethanol extract of all three samples shows maximum activity against both bacterial strains. Ethanol is an organic chemical compound whose bactericidal and antifungal effects are very much understood, and being a highly polar solvent, it can extract the therapeutically active compound from the drug samples, thus exhibiting its antibacterial ability. The other two extracts, chloroform and aqueous extract being less polar, shows less sensitivity against both bacteria. This viewpoint can be explained in Ayurveda also. In various Ayurvedic

formulations in, such as asava, arishtas and syrups, alcohol forms an important part. [10] Thus, Alambusha leaf can be prepared into these formulations and made available for treating bacterial infections. The study proved that the leaf ethanolic sample shows good sensitivity against the bacteria.

**Dose-Dependent Activity**

It was found that the volume of extracts (25 microliters, 50 microliters, 75 microliters, 100 microliters) taken at a fixed concentration of 20 mg/ml gets increased as the Zone of Inhibition also gets increased, thus showing high antibacterial sensitivity. This may occur as the volume of the extract grows, and the amount of extract to get into action in the Agar plates containing bacteria also increases. Thus, getting more value for Zone of Inhibition[11, 12].

## CONCLUSION

The present study highlights the antibacterial potentiality of Alambusha. All the plant samples, leaf, root and leaf, and root combination show some antimicrobial activity with varying results. Among all the plant samples, the leaf sample shows the maximum Zone of Inhibition against both the strains selected for the study and that too highest in *Pseudomonas aeruginosa*. A root sample against both bacteria follow this. And the combination sample shows the least sensitivity against both bacteria. Among all three extracts, the ethanol extract of all three samples is known to show maximum activity (Zone of Inhibition) against both bacterial strains. The other two extracts, chloroform and aqueous extract being less polar, shows less sensitivity against both bacteria. This shows the polarity dependence of extracts. Thus, through this study, it can be demonstrated that use of folk medicine can be used as an efficient replacement for combating various pathogenic microorganisms.

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