
ORIGINAL ARTICLE

Antibacterial Activity of Selected Leaf Extracts against *Bacillus* Sp.

A.S. Wabale, M. N. Kharde and V. S. Gulve

Post graduate Department of Botany,
Padmashri Vikhe Patil College of Arts, Science and Commerce,
Pravaranagar- 413 713
Email: dranilwabale@rediffmail.com

ABSTRACT

The bacterium affects the seed germination and plant development which leads to great economic loss mostly to the farmers. Thus to get more yield & to fulfill the need of over increasing population & to save farmers from huge economic loss, the studies on seed borne bacteria & their control using eco-friendly & natural antibacterial substances such as the plant extract is very much required. Methanolic extracts from the leaves of selected plants was used to test the inhibitory effect against the gram +ve bacteria i.e. Bacillus in in vitro conditions. The investigations on phytochemical characteristics analysis showed that crude form of authentic botanicals prevents diseases. The action of methanolic extracts of Parthenium hysterophorous, Azadirachta indica, Nerium oleander and Annona squamosa against Bacillus sp. has demonstrated the great potential of the plants as a source of an antibacterial agent. The plant extracts can be used in preparation of useful drugs/medicines for treating bacterial diseases on plants as well as on human beings.

Keywords: Methanolic extracts Parthenium hysterophorous, Azadirachta indica, Nerium oleander, Annona squamosa.

Received 12.08.2015 Accepted 07.09.2015

©2015 Society of Education, India

How to cite this article:

A.S. Wabale, M. N. Kharde and V. S. Gulve. Antibacterial Activity of Selected Leaf Extracts against *Bacillus* Sp. Adv. Biores., Vol 6 [5] September 2015:58-61. DOI: 10.15515/abr.0976-4585.6.5.5861

INTRODUCTION

Bacillus is a germ positive, soil dwelling and rod shaped bacterium belonging to family Bacillaceae. *Bacillus* occurs naturally in the gut of caterpillars of different types of moths and butterflies as well as on the leaf of various plants. Over 90 species of naturally occurring insect specific (entomopathogenic) bacteria have been isolated from the insects, plants and soil but only a few have been studied intensively. Different varieties of this bacterium produce a crystal protein that is toxic to specific group of insect, plants and animals. This bacterium also affects the seed germination and plant development. The seeds stored traditionally by farmers are associated with many dormant bacteria. These get activated when the seed are sown and shows their efficacy, symptoms. Due to these the seedlings get affected and shows unhealthy, diseased symptoms but as the cultivated area is more some of the plants show normal growth. Thus the effect of bacterium is not visualized by farmers. It leads to great economic loss mostly to the farmers [1].

To get more yield & to fulfill the need of over increasing population & to save farmers from huge economic loss every year, the studies on seed borne bacteria & their control using eco-friendly & natural antibacterial substances such as the plant extract is very much required. These compounds are cheap & easily available to the growers of the crop and are found by earlier researchers to have similar effects on the bacterium as that of the unaffordable chemical bactericides have [2].

On the other hand the chemical bactericide adversely affects the useful micro organisms present on the plant body and in environment. This has been recently proved that they are hazardous to plants, animals & also to human being. The bactericides & pesticides that are sprayed on the crops are being consumed by people every day resulting in the bioaccumulation of such compounds in human body and forming several disorders after reaching critical accumulation [3].

The present paper deals with the study of antibacterial activities of the leaf-extract of the various plants *in vitro* conditions.

MATERIALS AND METHODS

Fresh, healthy leaves of *Parthenium hysterophorous*, *Azadirachta indica*, *Nerium oleander* and *Annona squamosal* were used to prepare the plant extract. About 50 gm of fresh leaves were washed, blotted using blotter paper and crushed in mortar using pestle in 200 ml of methanol. The extract was then filtered through double layered muslin cloth. The filtrate was kept at room temperature for 24 hours for the evaporation of methanol and settlement of extract. The extract was collected in conical flask & was autoclaved for 20 minutes at 15 lb pressure & 121^o C temperatures. Such extract was used for inoculation/treatment. Simultaneously Petri-plates, NA medium, forceps, tips, conical flask, measuring cylinder, distilled water were also autoclaved.

Bacillus strain were inoculated in nutrient broth and allowed to form dense culture. 0.1 ml of broth was then spread on Petri-plates containing NA media using a sterile glass spreader. The wells were prepared using alcohol sterilized borer (8mm diameter). Then 15 µl of plant extract was added into each well using micropipette. Simultaneously streptomycin was added into separate Petri-plate to treat as a control. After 24 hours of incubation at 37^oC, the diameter of inhibition zone was determined. Control measurement was carried out using antibiotics (streptomycin) [4,5].

RESULTS AND DISCUSSION

The result of antibacterial activities of various methanolic extracts from the leaves of *Parthenium hysterophorous*, *Azadirachta indica*, *Nerium oleander* and *Annona squamosa* showed activity against *Bacillus subtilis*. The methanolic extracts of above mention plants were tested against gram positive bacterial strains. The concentrations of methanolic extracts had inhibitory effects towards the bacterial strains. The zone of inhibition of *Parthenium hysterophorous* showed maximum i.e. 21 mm and *Azadirachta indica* showed minimum i.e. 14 mm inhibition. Second best in inhibiting the bacteria was *Annona squamosa* with 19 mm inhibition and *Nerium oleander* with 17 mm stood next to it.

All plant parts synthesize some chemicals in themselves which metabolize their physiological activities. These phytochemicals are used to cure the disease in herbal and homeopathic medicine. Nowadays, most of the people prefer to use the traditional methods to cure general diseases. The various phytochemical compounds (secondary metabolites like glycosides, flavonoids, tannins, steroid, phenols) detected are known to exhibit medicinal activity as well as physiological activity. Glycosides are nonvolatile and lack fragrance and they serve as defense mechanisms against *Bacillus sp.* Flavonoids are widely distributed group of polyphenolic compounds, characterized by a common benzopyrene ring structure. The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatoxins, viruses and tumors. Flavonoids reduced cancers by interfering with the enzymes that produce estrogen. Tannins have been traditionally used for protection of inflamed surfaces of the mouth and treatment of catarrh, wounds, hemorrhoids and diarrhea. Plant tannins have been recognized for their pharmacological properties and are known to make trees and shrubs. Growth well of all the three gram negative bacteria were controlled by both the acetone and methanol extracts. Inhibition zone was developed.

Table 1: Effect of Antibiotic-Streptomycin/Plant extracts on growth of *Bacillus subtilis*

Sr. No.	Name of Antibiotic/Plant extracts	Zone of inhibition (mm)
1.	Streptomycin	Complete inhibition
2.	<i>Parthenium hysterophorous</i>	21 mm
3.	<i>Azadirachta indica</i>	14 mm
4.	<i>Nerium oleander</i>	17 mm
5.	<i>Annona squamosa</i>	19 mm

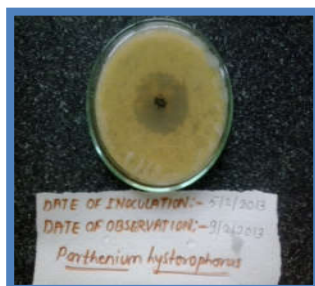


Photo showing the *in vitro* antibacterial activity of methanolic extract of *P. hysterophorus* on *Bacillus sp*



Photo showing the *in vitro* antibacterial activity of Antibiotic (*streptomycin*) on *Bacillus sp.*



Photo showing the *in vitro* antibacterial activity of methanolic extract of *A. indica* on *Bacillus Sp.*

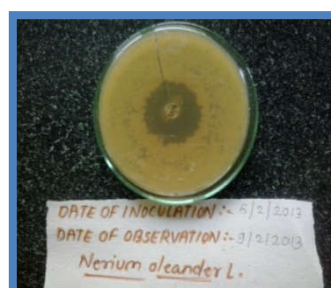


Photo showing the *in vitro* antibacterial activity of methanolic extract of *N. oleander* on *Bacillus Sp.*



Photo showing the *in vitro* antibacterial activity of methanolic extract of *A. squamosa* on *Bacillus Sp.*

Fig 1: Antibacterial Effect of Various plant extracts

CONCLUSIONS

The bacterium also affects the seed germination and plant development. This leads to great economic loss mostly to the farmers [1]. Thus to get more yield & to fulfill the need of over increasing population & to save farmers from huge economic loss, the studies on seed borne bacteria & their control using eco-friendly & natural antibacterial substances such as the plant extract is very much required. These compounds are cheap & easily available. On the other hand the chemical bactericide adversely affects the useful micro organisms present in environment. This has been recently proved that they are hazardous to plants, animals & also to human being.

Methanolic extracts from the leaves of selected plants was used to test the inhibitory effect against the gram +ve bacteria i.e. *Bacillus* in *in vitro* conditions. The investigations on phytochemical characteristics

analysis showed that crude form of authentic botanicals prevents diseases. The action of methanolic extracts of *Parthenium hysterophorous*, *Azadirachta indica*, *Nerium oleander* and *Annona squamosa*. against *Bacillus* sp. has demonstrated the great potential of the plants as a source of an antibacterial agent.

The plant extracts can be used in preparation of useful drugs/medicines for treating bacterial diseases on plants as well as on human beings.

REFERENCES

1. Agarwal VK and JB Sinclair. (1996). Principles of Pathology. 2nd ed, CRC Press, Inc., Boca Raton, Fl. 539 pp.
2. Barrow GI, Feltham RKA (1993). Characters of Gram-negative bacteria. In: Cowan and Steel Manual for Identification of Medical Bacteria. 3rd edition, Cambridge University Press, pp. 94-149.
3. Ogbolu DO, Daini OA, Terry Alli OA, Adeladan O, Salako AO., Olusoga-Ogbolu FF, Oni AA (2010). In-vitro resistance of Gram-negative enteric bacilli from wound infections to honey. *Afr. J. Clin. Exper. Microbiol.*, **11**(3): 179-181.
4. Bonjar GHS and Farrokhi PR, (2004) Anti*Bacillus* activity of some plants used in traditional medicine of Iran. *Niger. J. Nat. Prod. Med.*, **8**, 34-39.
5. Abo K A, Adeyemi A A and Jegede I A, (2000) Spectrophotometric estimation of anthraquinone content and antimicrobial potential of extracts of some *Cassia* species used in herbal medicine in *Ibadan*. *Sci. Forum.*, **3**(2), 57-63.