International Journal of Education, Modern Management, Applied Science & Social Science (IJEMMASSS)
ISSN : 2581-9925, Impact Factor: 6.882, Volume 04, No. 03(II), July - September, 2022, pp. 19-22

DETERMINATION OF ANTIMICROBIAL ACTIVITY USING AZADIRECTA INDICA EXTRACT

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ABSTRACT

Plants are very important part of mankind from history these are used in various kind of medicines. There are different kind of medicinal plants which are in use from years to treat infections and diseases. This study shows the antimicrobial activity of Neem (Azadirachta indica) which was evaluated against two gram-negative bacteria Escherichia coli and Salmonella typhi (pathogenic bacteria) and gram-positive bacteria (Bacillus subtilis). Azadirachta indica green leaf and bark was collected from the local fields of Medicinal Garden in School of Pharmaceutical Studies, Faculty of Heath Sciences, Newai and clear cultures of the test micro-organisms used for antimicrobial study were provided by the Dr. K. N. Modi University, Newai Rajasthan. All the test microorganisms were selected for their antibacterial activity with leaf and bark extract of Azadirachta indica by agar well diffusion method. Leaf and bark extracts of Azadirachta indica showed good inhibition zone against the gram-positive and gram-negative bacteria.

Keywords: *Azadirachta Indica, Antimicrobial Activity, Pathogenic Bacteria, Leaf and Bark Extract.*

Introduction

Medicinal plants are a part of human society to fight with diseases, Plants are helping us to recover from dieseases since human species were living in forests till today. Azadirachta indica is well known in India and its nearby countries from more than 2000 years as one of the most useful medicinal plants having a wide spectrum of biological activity against microbial species. In sanskrit Neem is known as 'Arishtha' which means 'reliever of sickness' and hence is considered as Sarbaroganibarini'. A. Indica is also known as 'village dispensary' in Indiaan villages. The neem tree has been described as A. indica as early as 1830in an article written by De Jussieu [1,11].

- Indica is used in traditional medicine as a source of many therapeutic agents in the Indian peoples now Neem spreading roots to the developed countries. Many studies done on neem by researchers, have showed that it contains various active substances with multiple medicinal properties [2,]. Neem has very good potential of treating as much as other botanical plants [12]. Aqueous extract of neem leaf has a good therapeutic potential as antihyperglycemic agent in IDDM and NIDDM [3,4]. Neem leaves has antibacterial properties and could be used for controlling airborne bacterial contamination in the residential premise [4,5]. An article written by *Gbotolorun* has shown that administration of neem flowers alcoholic extract which found disrupts the estrous cycle in Sprague Dawley rats and causes some blockage in ovulation and has the potential of an ideal antifertility agent [6]. Because of their biodegradability, low persistence, low toxicity to organisms other than the target, affordability, and ease of supply, plant-based products are becoming more and more popular. Following is the taxonomic position of neem: [1]

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Table 1: Classification of Azadirecta Indica

Order	Rutales
Suborder	Rutinae
Family	Meliaceae (mahogany family)
Subfamily	Melioideae
Tribe	Melieae
Genus	Azadirachta
Species	indica

Materials and Methods

Collection of Samples

Azadirachta indica leaves and barks were collected from the fields of Medicinal Garden in School of Pharmaceutical Studies, Faculty of Heath Sciences, Dr. K. N. Modi University, Newai Rajasthan. It was authentified by the botany department of Rajasthan university, Jaipur. The plant material collected was healthy and free from any deformities.

Extract Preparation

The harvested plant material was taken to the lab for additional processing. The plant components were cut into small bits and then processed through a mixing blender to create powder. The powder is then removed from the sieve to obtain particles of the same size. The powder needs to be stored aseptically in an airtight container in a dry location. Care is taken when choosing solvents for extraction so that they can meet regulatory requirements and be extractable. A conical flask filled with 200 ml of distilled water and 25 g of powder are correctly weighed, transferred, and thoroughly mixed with the water. The flask containing the mixture of powder and water is put on room temperature on aseptic condition for 7 to 8 days, extracted and filtered using muslin cloth and Whatman filter paper. The purified extract was produced in the form of supernatant after centrifuging the filtered liquid material for five minutes at 4,000 rpm. For further use, this pure extract was kept at 4°C [7,13].

Isolation of Test Organisms

The Faculty of Health Sciences at Dr. K. N. Modi University, Newai, Rajasthan, provided pure cultures of the test organisms used for the antimicrobial investigation. Nutrient agar slant was used to cultivate every test organism. Prior to usage, the cultures were stored at 4°C and subculture frequently. Escherichia coli, Salmonella typhi, and Bacillus subtilis are among the gram-negative and gram-positive strains of bacteria, respectively.

Antibacterial Activity

By using the agar well diffusion method, all test organisms were evaluated for their antibacterial activity against Azadirachta indica leaf and bark extract. Antimicrobial susceptibility testing became important with the advent of several antimicrobials. For this, a plate that had just been spread with the test organism on it was given time for the antimicrobial agent to diffuse into the medium and interact. Muller-Hinton agar was used to test for antibacterial activity [8].

Preparation of Stock Solution

Stock solution of the extract was prepared to perform the antimicrobial activities to the selected cultures. For the preparation of the stock solution, 1 g of the extract in which 0.5 g Leaf extract and 0.5 g bark extract was accurately weighed and dissolved in 10 ml Dimethyl Sulfoxide (DMSO); giving concentration of the stock solution as 100 mg/ml. This solution was then centrifuged and supernatant liquid was collected in a separate test tube, covered with paraffin wax and stored at 4°C for further use [9].

Agar Well Diffusion Method

The Muller-Hinton agar plates were prepared for the antibacterial activity. We took 0.1 ml of the freshly prepared 18 h old broth culture was spread on the respective media. After spreading the culture, circles of 6 mm in diameter were made at the centre of the plate by using sterile cork borer. The circles were opened by the help of sterilized forceps. Then 100 µL of original stock solution was added by using micropipette in each well. The final concentration was found in the well was 1 mg per ml. The extract started diffuse from higher to lower concentration hence the prepared plates were left at RT (room temperature) for 30 minutes and then stored in incubator at 37°C for 24 hours [10].

Results and Discussion

Nutrient agar plates were cultured and observation were found, anti-bacterial activity of extract of Azadirachta Indica leaf and bark was evaluated against both gram + Ve and gram - Ve bacteria. Different extracts of Azadirachta indica Leaf and bark showed high inhibition zone against B. subtilis, while E. coli and S. typhi are less susceptible to neem extract.

Table 2: Antimicrobial Activity of Leaf and Bark Extract of Azadirachta Indica

Test Organism	Zone of inhibition
Escherichia coli	10 mm
Salmonella typhi	9 mm
Bacillus subtilis	16 mm

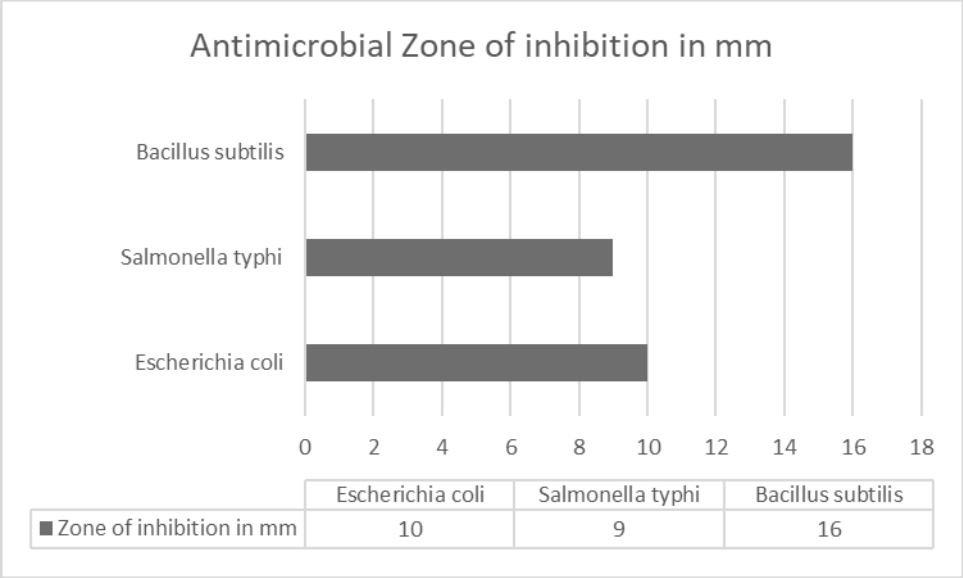

Chart 1: Antimicrobial Zone of Inhibition in mm

The findings of this study coincide with the observations of several researchers. Oil extracted from the leaves, seeds and bark shows a wide spectrum of antibacterial activity action against both gram + Ve and gram - Ve bacteria. Koona and Budida mentioned in their article the antimicrobial activity of the seed oil activity against a various pathogen.

Conclusion

Azadirachta indica extract is an important source of compounds having anti-microbial, anti-oxidant, anti-tumour, anti-malarial, anti-fungal, anti-inflammatory and anti-viral properties. The results of this research work indicated that using different parts of A. Indica had very beneficial effects in controlling the pathogenic microbial organisms and thus it can be used in different therapeutic formulations in upcoming future.

Acknowledgements

Authors are highly thankful to The Principal and Head Dr. Shailesh Sharma, School of Pharmaceutical Studies, Faculty of Health Sciences. Authors are also thankful to Dean Academics and registrar of university for making availability of microorganisms for providing bacterial cultures for the study. We are very thankful to Dr. K. N. Modi University, Newai, Rajasthan for providing laboratory facility and infrastructure.

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