

**INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY,
BIOLOGY AND CHEMISTRY****Research Article****In-vitro Study of *Azadirachta indica* Leaf Extract on
Staphylococcus Sps. Isolated from old books****EVN. Raju* and G. Divakar**Department of Biotechnology & Microbiology, Acharya & B.M.Reddy College of
Pharmacy, Soldevanahalli, Hesaraghatta, Bangalore, Karnataka, India.**ABSTRACT**

Micro – organisms are omnipresent. Thus, the pages on which we pen down our imagination are also the habitat for these microbes, with this in mind ,we isolated micro – organisms from a very old book of 15 years from our College library. By gram – staining and observation under the microscope, Cocci were observed. On performing various biochemical tests such as catalase , oxidase, starch hydrolysis, gelatin, we identified the organisms to be a species staphylococcus sps . Antibacterial test were performed in – vitro using *Azadirachta indica* leaf extracts to study its role on the inhibition of this pathogen. A standard borewell method was employed.

Keywords: *Azadirachta indica* , Staphylococcus ,Catalase test, Oxidase test, Old books.

INTRODUCTION

Microorganisms like bacteria are omnipresent, which exist either as Cocci or rods. Among the Cocci, Staphylococcus sps., E.coli sps., Bacillus sps. And Diplobacillus sps. are some common examples. Staphylococcus sps. is a gram-positive bacterium that appears as a grape like clusters. The Staphylococcus genus has about thirty-three species. Most of them are harmless and reside normally on the skin and mucous membranes of humans and other organisms. Also found worldwide, they are a small component of soil microbial flora. Staphylococcus can cause a wide variety of diseases in humans and other animals through either toxin production or invasion. Toxins from this organism is a common cause of food poisoning, When food is improperly stored.

Azadirachta indica (*Meliaceae*). is an ancient medicinal plant native to India Sri Lanka Pakistan and Bangladesh. Common folk in India uses this plant for healing skin diseases. *Azadirachta indica* is an indigenous medical plant. It has been prescribed in Ayurveda as an alterative, anthelmintic, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation and asthma. In Cameroon, the whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of

this plant are important in the management of various ailments.

MATERIALS AND METHODS**MATERIALS**

Petridish, cotton swabs, boiling tube, NA media , gelatin, iodine, inoculation, alcohol, spirit lamp, etc.

METHODS

Using basics concepts of microbiology. We prepared agar media, blood agar media as a nutrient media (S.S.FINECHEM. LIMITED, MUMBAI.) bacteria needs agar media i.e. Na media for its growth .Now we isolated the bacteria by swabbing with the cotton swabs from middle of the book and applied it on NA media plate .Now we incubated it for 18 hour at 37^oC and we observed white coloured colonies on NA media plates .We sub cultured the already prepared bacteria and thus we obtain NA media plate containing a pure culture. we did gram staining using the gram's iodine , crystal violet stain , saffranin , alcohol and bacterial pure culture and we performs biochemical test such as starch hydrolysis , gelatin test , indole test , methyl red test , catalase test and oxidase test.

Observation

The results for various biochemical tests are given in the table:1

RESULTS

The bacteria which were isolated from old books were found to be Cocci. These were confirmed to be *Staphylococcus* sps. Based on biochemical tests such as catalase, oxidase, methyl red and gelatine test which were positive, whereas the starch hydrolysis and indole tests showed a negative results, this confirms the presence of *Staphylococcus* sps.

The antimicrobial effect using the crude extract of *Azadirachta indica* was conducted. Crude extract which was extracted from organic solvent showed an antibacterial property against pathogenic bacteria.

CONCLUSION AND DISCUSSION

We concluded that the organism isolated from the old books was found to be *Staphylococcus* in-vitro antibacterial tests using a crude juice of *Azadirachta indica* on *Staphylococcus* showed a clear zone of inhibition. Pathogenic bacteria like *Staphylococcus* can cause a wide variety of diseases in humans and other animals through either toxin production or invasion. Staphylococcal toxins are a common cause of food poisoning, as it can grow in improperly-stored food. In year 1964 two scientists namely Evenleigh and Brewer observed bacteria isolated from old papers from Canadian paper Mill. Our study concludes that this 'medicinal plant' showed an antibacterial property against pathogenic bacteria.

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Table 1:

Tests	Inference
Starch Hydrolysis	-ve
Gelatine Hydrolysis	+ve
Indole Test	-ve
Catalase Test	+ve
Oxidase Test	+ve
Methyl Red Test	+ve

Note : Gram – staining and biochemical tests indicated that the Micro -organism was *Staphylococcus* sps.



Fig. 1: Bacterial Culture



Fig. 2: Pure Bacterial Culture

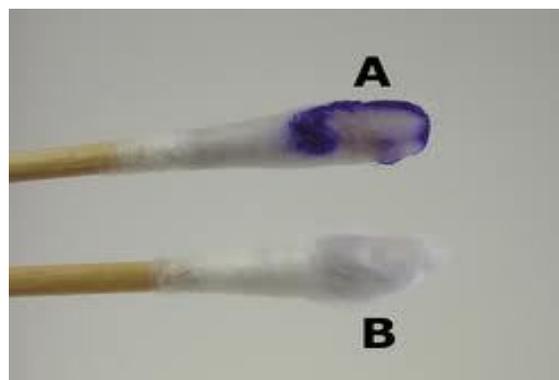


Fig. 3: Oxidase Test (A is Positive)



Fig. 4: Catalase test (Bubbles indicates positive)



Fig. 7: Gelatin test (slant liquid indicates positive)



Fig. 5: Starch hydrolysis test



Fig. 8: Indole test (negative)



Fig. 6: M.R Test (pink colour indicates positive)



Fig. 9: Crude Juice of *Azadirachta indica*

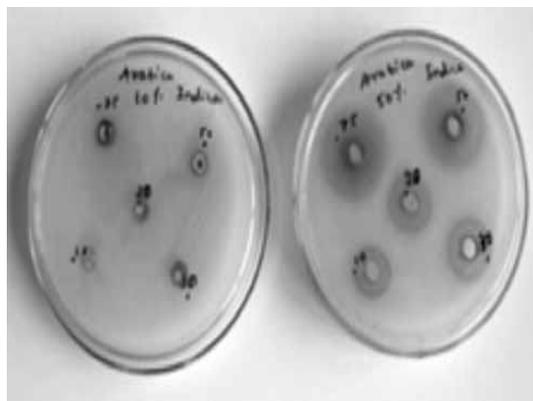


Fig. 10: Anti bacterial test

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Although a few in vitro studies have tested Neem leaf extract on *S. aureus*, there are none done on Methicillin-resistant Staphylococcus aureus (MRSA) despite the fact that it is being marketed for such purposes. Objectives: This study aims to determine if Neem leaf extract (*Azadirachta indica*) has antibacterial properties against Methicillin-sensitive and Methicillin-resistant Staphylococcus aureus and to compare the anti- The petroleum ether, methanol and aqueous extracts of the seeds of *Helianthus annuus* (Asteraceae), leaves of *Azadirachta indica* (Meliaceae), bulbs of *Allium cepa* (Liliaceae) and that of the seeds of *Portulaca oleracea* (Portulacaceae) were screened for their anti-microbial activity using the Cup plate agar diffusion method. Biological activities and medicinal properties of neem (*Azadirachta indica*). Kausik Biswas, Ishita Chattopadhyay, Ranajit K. Banerjee* and Uday Bandyopadhyay. Department of Physiology, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Kolkata 700 032, India. isolated from the bark of *Melia azadirachta*, demonstrated strong antitumour effect with complete regression of the tumours, when administered in mice at a daily dose. Although a large number of studies have been carried out on various biological activities of neem extracts and some of the isolated compounds in several animal models, a few reports are available on clinical studies with the extracts or the compounds and their medicinal applications. Neem extract. *Staphylococcus* Sps. Isolated from old books. EVN. Raju* and G. Divakar Department of Biotechnology & Microbiology, Acharya & B.M.Reddy College of Pharmacy, Soldevanahalli, Hesaraghatta, Bangalore, Karnataka, India. ABSTRACT Micro organisms are omnipresent. Thus, the pages on which we pen down our imagination are also the habitat for these microbes, with this in mind, we isolated micro organisms from a very old book of 15 years from our College library. Antibacterial tests were performed in vitro using *Azadirachta indica* leaf extracts to study its role on the inhibition of this pathogen. A standard borewell method was employed. Keywords: *Azadirachta indica*, *Staphylococcus*, Catalase test, Oxidase test, Old books.