



## IN-VITRO STUDY OF ALLIUM SATIVUM FRESH JUICE ON BARBER SHOP UTENSILS MICROBIAL ISOLATES

E.VENKATA NAGA RAJU\* Dr.G. DIVAKAR

1. DEPARTMENT OF BIOTECHNOLOGY & MICROBIOLOGY, ACHARYA & B.M.REDDY COLLEGE OF PHARMACY,  
BANGLORE

Mail id: [evnrazbiotech@gmail.com](mailto:evnrazbiotech@gmail.com)

### ABSTRACT

Microorganisms are omnipresent. Thus, the places on which we pen down our imagination are also the habitat for these microbes, with this in mind, we screened Eleven barber shops utensils like combs, brush, clippers and apron in diverse places of Bangalore. A variety of selective and differential microbial media were used for presumptive identification of contaminating microorganisms which were authenticated by Gram – staining and observation under the microscope. On performing various biochemical tests such as Catalase, Oxidase, Starch hydrolysis, Gelatin, Coagulase test and Mannitol fermentation test we identified the organisms as *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecium*. Antibacterial tests were performed in – vitro by using *Allium sativum* fresh juice to study its role on the inhibition of these pathogens.

**Keywords** *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecium* Catalase test, Oxidase test, Barbershop, *Allium sativum*.

### INTRODUCTION

Healthcare is one of the most important aspects of all human beings aimed at improving the quality of life, since sound health is essential for the strength and prosperity of a nation. In developing countries, infections remain the main cause of morbidity and mortality in humans where it is mostly associated with poverty and overcrowding.

Barbers are important professionals in the community. It has been shown that barbering operations include cutting, face and scalp massaging, nail trimming, pedicure, manicure and shampooing/dyeing of hair can transmit various health hazards communicable diseases, like ringworm (via direct contact), infestation of head, scabies (via contaminated towels, combs and aprons), hepatitis, HIV (via contaminated blades and clips), Skin Infections, Toxic Shock Syndrome, Osteomyelitis, Gastroenteritis and Pneumonia which are caused by *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecium* (via contaminated knife, blades and combs).

For example, where instruments and materials used on client are not sterilized or are not properly handled and used hygienically, sharp instruments such as razors, clippers and scissors may become contaminated if they pierce the skin of infected person (clippers can accidentally pierce and penetrate the skin) and it should be noted that blood and body fluids do not have to be visible on instruments or working surfaces for infection to be transmitted and both clients and operator are at risk. *Allium sativum* (Amaryllidaceae) is a well – known spice which is used in foods all over the world and its antibacterial effect against food borne pathogens like *Salmonella*, *Shigella* and *S. aureus* has been recognized (Teferi and Hahn, 2002).

The aim of this study was to assess the microbiological hazards and practices linked with their transmission in the barbering practice and Antibacterial activity of *Allium sativum* fresh juice to study its role on the inhibition of this pathogen.

## MATERIALS AND METHOD

### MATERIALS

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

### Collection and isolation of sample

Samples were collected from barber shops in diverse places ( Solddevanahalli , Chikkabanavara, Devasandra, K.R.puram, Tannary road, Yashwanthpur and at Tumkur in and around Bangalore, Karnataka, India. The samples were labeled after collected. These were spread onto isolation media and incubated at 37oC for 18 hours .

**Table -1:Tabulation for Samples**

| S.NO | DESIGNATION OF SAMPLE | SAMPLE COLLECTED AREA |
|------|-----------------------|-----------------------|
| 1    | ACP-1                 | Shivaji Nagar         |
| 2    | ACP-2                 | Tannery Road          |
| 3    | ACP-3                 | Tannery Road          |
| 4    | ACP-4                 | Tannery Road          |
| 5    | ACP-5                 | Solddevanahalli       |
| 6    | ACP-6                 | Chikka Banavara       |
| 7    | ACP-7                 | K.R.Puram             |
| 8    | ACP-8                 | Tin Factory           |
| 9    | ACP-9                 | Tumkur Dairy          |
| 10   | ACP-10                | Tumkur Dairy          |
| 11   | ACP-11                | Yashwanth Pura        |

**ACP DENOTES ACHARYA COLLEGE OF PHARMACY**

### METHOD

Using basics concepts of microbiology. Samples were collected from Eleven barber shops in diverse places of Bangalore. To determine the types of microorganisms present on the comb, brush, clippers and aprons were sampled with a moistened sterile cotton swab. After taking each swab, the swab stick was placed back into the casing to avoid contamination and was labelled appropriately. A variety of selective and differential microbial media were used for presumptive identification of contaminating microorganisms. Gram staining, microscopic examination and confirmatory biochemical tests were performed to further identification.

We prepared agar media, blood agar media as a nutrient media (S.S.FINECHEM. LIMITED, MUMBAI.) for microbial growth .Now we isolated the microorganisms by swabbing with the cotton swabs from utensils like combs, brush, clippers and apron and applied it on NA media plate .Now we incubated it for 18 hour at 37°C and we observed white coloured colonies on NA media plates .We sub cultured the colonies on same media to obtain pure cultures. we did gram staining using the gram's iodine , crystal violet stain , saffranin , alcohol and microbial pure culture and we performed biochemical test such as starch hydrolysis , gelatin test , indole test , methyl red test , catalase test ,coagulase and Mannitol fermentation test .

**Table -2:Tabulation for results of colony characteristics**

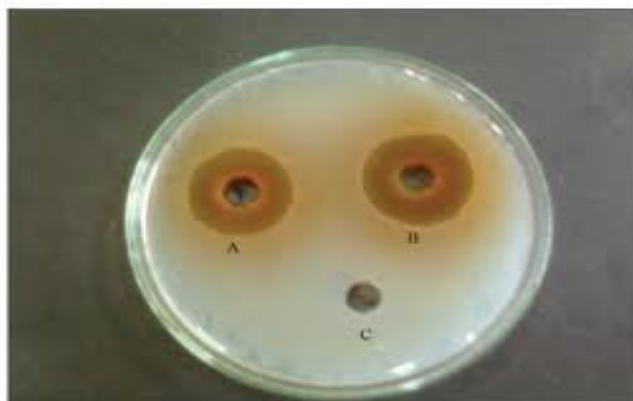
| STRAIN NO. | COLONY SURFACE | COLONY COLOUR | VISUAL CHARACTERISTICS | SHAPE OF THE COLONY | HEIGHT OF THE COLONY |
|------------|----------------|---------------|------------------------|---------------------|----------------------|
| G.D-1      | Smooth         | Brown         | Opaque                 | Irregular           | Raised               |
| G.D-12     | Smooth         | Off white     | Opaque                 | Circular            | Raised               |
| G.D-20     | Smooth         | Brown         | Opaque                 | Irregular           | Flat                 |
| G.D-30     | Smooth         | Off white     | Opaque                 | Irregular           | Raised               |
| G.D-32     | Smooth         | Brown         | Opaque                 | Circular            | Raised               |
| G.D-42     | Smooth         | Off white     | Opaque                 | Circular            | Flat                 |
| G.D-50     | Smooth         | Off white     | Opaque                 | Irregular           | Raised               |
| G.D-55     | Smooth         | Brown         | Opaque                 | Circular            | Raised               |
| G.D-58     | Smooth         | Off white     | Opaque                 | Circular            | Flat                 |
| G.D-59     | Smooth         | Off white     | Opaque                 | Circular            | Raised               |
| G.D-65     | Smooth         | Off white     | Opaque                 | Circular            | Flat                 |
| G.D-66     | Smooth         | Off white     | Translucent            | Irregular           | Raised               |
| G.D-69     | Smooth         | Off white     | Translucent            | Irregular           | Raised               |
| G.D-72     | Smooth         | Off white     | Translucent            | Circular            | Flat                 |
| G.D-78     | Smooth         | Brown         | Translucent            | Circular            | Raised               |
| G.D-80     | Smooth         | Brown         | Opaque                 | Irregular           | Flat                 |
| G.D-88     | Smooth         | Brown         | Opaque                 | Irregular           | Raised               |
| G.D-97     | Smooth         | Brown         | Translucent            | Irregular           | Flat                 |
| G.D-100    | Smooth         | Brown         | Translucent            | Irregular           | Flat                 |

**Figure 1. Pure culture****Figure 2: Antimicrobial Activity of Allium sativum fresh juice.**

(a). Antimicrobial Activity of Allium sativum fresh juice on Staphylococcus aureus pure culture



(b) Antimicrobial Activity of *Allium sativum* fresh juice on *Bacillus subtilis* pure culture



(c). Antimicrobial Activity of *Allium sativum* fresh juice on *Enterococcus faecium* pure culture



### Identification of Bacteria

The isolated bacteria were identified based on cellular morphology, growth condition, grams staining, endo spore staining, capsule staining and biochemical tests (Pokorny, M., L.J. Vitale;1979).

**Table -3: Tabulation for results of Staining Techniques.**

| <b>STRAIN NO.</b> | <b>GRAM STAINING</b> | <b>MORPHOLOGY (BACILLUS/COCCI)</b> | <b>ENDOSPORE STAINING</b> | <b>CAPSULE STAINING</b> |
|-------------------|----------------------|------------------------------------|---------------------------|-------------------------|
| <b>G.D-1</b>      | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-12</b>     | Negative             | Cocci                              | Positive                  | Positive                |
| <b>G.D-20</b>     | Negative             | Cocci                              | Positive                  | Positive                |
| <b>G.D-30</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-32</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-42</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-50</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-55</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-58</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-59</b>     | Negative             | Cocci                              | Positive                  | Positive                |
| <b>G.D-65</b>     | Negative             | Cocci                              | Positive                  | Positive                |
| <b>G.D-66</b>     | Negative             | Cocci                              | Positive                  | Positive                |
| <b>G.D-69</b>     | Negative             | Cocci                              | Positive                  | Positive                |
| <b>G.D-72</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-78</b>     | Positive             | Cocci                              | Positive                  | Positive                |
| <b>G.D-80</b>     | Positive             | Bacillus                           | Positive                  | Positive                |
| <b>G.D-88</b>     | Positive             | Bacillus                           | Positive                  | Positive                |
| <b>G.D-97</b>     | Positive             | Bacillus                           | Positive                  | Positive                |
| <b>G.D-100</b>    | Positive             | Bacillus                           | Positive                  | Positive                |

**Table -4: Tabulation for results of Various Biochemical tests**

| S.No. | SAMPLES | INDOLE | MR  | VP  | AMYLASE | NITRATE | OXIDASE | CATALASE | UREASE | GELATINASE | CASEIN |
|-------|---------|--------|-----|-----|---------|---------|---------|----------|--------|------------|--------|
| 1     | G.D-1   | -Ve    | -Ve | -Ve | +Ve     | -Ve     | +Ve     | +Ve      | -Ve    | +Ve        | +Ve    |
| 2     | G.D-12  | -Ve    | -Ve | +Ve | +Ve     | +Ve     | +Ve     | -Ve      | +Ve    | +Ve        | +Ve    |
| 3     | G.D-20  | -Ve    | +Ve | -Ve | +Ve     | +Ve     | +Ve     | -Ve      | +Ve    | +Ve        | +Ve    |
| 4     | G.D-30  | -Ve    | +Ve | -Ve | +Ve     | +Ve     | -Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 5     | G.D-32  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 6     | G.D-42  | -Ve    | -Ve | +Ve | -Ve     | +Ve     | +Ve     | +Ve      | -Ve    | +Ve        | +Ve    |
| 7     | G.D-50  | -Ve    | -Ve | +Ve | -Ve     | +Ve     | +Ve     | +Ve      | -Ve    | +Ve        | +Ve    |
| 8     | G.D-55  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 9     | G.D-58  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 10    | G.D-59  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 11    | G.D-65  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 12    | G.D-66  | -Ve    | -Ve | +Ve | -Ve     | +Ve     | +Ve     | +Ve      | -Ve    | +Ve        | +Ve    |
| 13    | G.D-69  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | +Ve        | +Ve    |
| 14    | G.D-72  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | -Ve        | +Ve    |
| 15    | G.D-78  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | -Ve        | +Ve    |
| 16    | G.D-80  | -Ve    | -Ve | +Ve | +Ve     | -Ve     | +Ve     | -Ve      | -Ve    | -Ve        | +Ve    |
| 17    | G.D-88  | -Ve    | -Ve | +Ve | -Ve     | -Ve     | +Ve     | +Ve      | -Ve    | -Ve        | +Ve    |
| 18    | G.D-97  | -Ve    | -Ve | +Ve | +Ve     | +Ve     | +Ve     | +Ve      | -Ve    | +Ve        | +Ve    |
| 19    | G.D-100 | -Ve    | -Ve | -Ve | -Ve     | +Ve     | -Ve     | +Ve      | -Ve    | +Ve        | +Ve    |

## RESULTS AND DISCUSSION

The microbial pure cultures which were isolated from utensils of barber shop like combs, brush, clippers and aprons found to be Cocci and Bacilli and were confirmed to be *Staphylococcus aureus*, *Bacillus subtilus* and *Enterococcus faecium*. Based on biochemical tests such as catalase, Mannitol fermentation test and coagulase test which were positive, whereas the starch hydrolysis, methyl red, gelatin test and indole tests showed a negative results.

The antimicrobial effect was conducted by using *Allium sativum* fresh juice. Fresh juice which was extracted from organic solvent showed an antibacterial property against pathogenic bacteria (Figure 2).

## Conclusion

We concluded that the Nineteen bacterial isolates were obtained (Table:3) from samples of ACP 1 to ACP 11 (Table:1) found to be *Staphylococcus aureus*, *Bacillus subtilus* and *Enterococcus faecium* Morphologically and biochemically. The colonies were subjected to Grams staining, capsule staining and endospore staining. The colonies which were positive for Grams staining, Capsule and endospore staining were considered for further studies (Table 3&4). and in-vitro antibacterial tests conducted on these isolates by using *Allium sativum* fresh juice showed a clear zone of inhibition. Pathogenic bacteria like *Staphylococcus aureus*, *Bacillus subtilus* and *Enterococcus faecium* can cause a wide variety of diseases from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia and sepsis. Hence our study clearly indicates that much effort has to be put in educating clients of barbershops and service providers about hazards inherent in barbering practice and the importance of putting preventive measures in place, and antibacterial activity of *Allium sativum* fresh juice.

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