



# A REVIEW ON ANTIMICROBIAL ACTIVITY OF INDIAN TRADITIONAL MEDICINAL PLANTS

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## ABSTRACT

This study was undertaken to identify anti-microbial activity of Indian traditional medicinal plants and their description, morphological characteristics, chemical constituents, and their uses such as antibacterial activity, antiviral activity, antifungal activity, antioxidant characteristics and that are effective against multiple human pathogens and to partially purify the active component through thin layer chromatography.

Antibacterial activity of selected plant extracts were assayed by agar cup diffusion. Minimum inhibitory concentrations were determined against all the pathogens. Sensitivity of the pathogens was also checked with four standard antibiotics. In addition, the stabilities of the active compounds were checked at different temperature and pH conditions.

Extracts were separated using TLC and relative mobilities of bioactive components were determined by contact bioautography. Ethanolic extracts of Amla (*Emblica officinalis*) fruit, Neem (*Azadirachta indica*) leaves, Aloe (*Aloevera*) leaves, Assam Tea (*Camellia sinensis assamica*) leaves and Clove (*Syzygium aromaticum*) buds were found to inhibit the growth of methicillin resistant *Staphylococcus aureus*, *Vibrio cholerae* and *Pseudomonas aeruginosa*. Bioactive components were stable over a range of pH values and temperatures. (Mehrotra et al., 2010)

## Key words:

- *Azadirachta indica*,
- *Aloe vera*,
- *Camellia sinensis assamica*,
- *Syzygium aromaticum*,
- *Staphylococcus aureus*,
- *Vibrio cholerae*,
- *Pseudomonas aeruginosa*

## INTRODUCTION

Since ancient time, naturally occurring plants have played an important role in the discovery of new therapeutic agents. Herbal medicines are becoming more and more popular. Among the entire flora 35,000 to 70,000 species have been used for medicinal purposes. Even today the WHO estimated that up to 80% of people still rely mainly on traditional medicines such as herbs for their remedies. Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. For the treatment of infectious diseases, search of substitutes from the nature to the antibiotics is becoming the prime need of the society in the present and the future. The progressive increase in the antibacterial resistance among the entire pathogen is critical concern for the people of developing world.

Ayurveda, the traditional Indian medicine (TIM) and traditional Chinese medicine (TCM) remain the most ancient yet living traditions. These are the two 'great traditions' with sound philosophical, experiential and experimental basis. Increased side effects, lack of curative treatment for several chronic diseases, high cost of new drugs, bacterial resistance and emerging diseases are some reasons for renewed public interest in complementary and alternative medicines.

**Infection:** Bacteria are one-celled organisms that do not have membranes binding their nuclear material (prokaryotes). This feature distinguishes them from protozoa which have a more complex cellular structure and a distinct nucleus (eukaryotes). Not all bacteria cause diseases. Bacteria are present in some fermented foods. Yogurt, for example, has *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria. The human mouth and intestines harbor over 400 different types of bacteria that produce some vitamins and ferment fiber to produce short-chain fatty acids.

List of common bacteria and some of their attributes

- *Staphylococcus* - normally found on the skin, but can cause boils and pimples.
- Methicillin-resistant *Staphylococcus aureus* (MRSA) is responsible for many
- *Escherichia coli* - normal inhabitant of the colon, hence called "coliform" bacteria
- *E. coli* O157:H7 is a virulent strain that produces toxins that can cause diarrhoea, abdominal pain, and even kidney failure. Chlamydia - a sexually transmitted disease (STD) caused by the bacterium *Chlamydia trachomatis*.
- *Salmonella* - frequent cause of food poisoning *Vibrio cholerae* - causes cholera, an infection of the small intestine characterized by watery diarrhoea
- *Treponema pallidum* - a spiral-shaped (spirochete) bacteria that causes syphilis.
- *Neisseria gonorrhoeae* - a Gram-negative coccus that causes gonorrhoea, one of the most common sexually transmitted diseases
- *Borrelia* - a spirochete transmitted by ticks that causes Lyme disease (borreliosis).
- *Mycobacterium tuberculosis* - cause of tuberculosis
- *Yersinia pestis* - causes bubonic plague, transferred by flea bites

- *Bacillus anthracis* - the organism that causes anthrax, characterized by black lesions.
- *Rickettsia* - a motile, Gram-negative bacterium that replicates only within the cytoplasm of cells and causes diseases such as typhus, rickettsialpox, and Rocky Mountain spotted fever. It is transmitted by the bites of insects such as ticks, fleas, and lice.

**Infections:** Infection involves interaction between the animal body (host) and the infecting microorganism. Infection and infectious disease have to be distinguished. The lodgement and multiplication of a parasite in or on the tissues of a host constitute infection. Infectious disease is a rare consequence of infection, which is a common natural event.

Infections are classified in various ways.

- Primary infection:** Initial infection with a parasite in a host.
- Re-infections:** Subsequent infections by the same parasite in the host.
- Secondary infections:** Infection caused due to a new parasite in a host whose resistance is lowered by a preexisting infectious disease.
- Focal infection:** Infection or sepsis at localized sites like appendix or tonsils. Generalized effects are produced.
- Cross-infection:** Host already suffering from a disease, a new infection is set up from another host or another external source.
- Endogenous infection:** Source of infection is from the host's own body.
- Exogenous infections:** Source of infection is from the external sources.

**Sources of Infection:** Infection-causing sources are various types and it includes humans, animals, insects, soil, water, and food.

**Allopathic treatment:** Allopathic treatment involves the use of antibiotics. Antibiotics are small molecules that kill or stop the growth of bacteria by blocking essential functions within the bacterial cell. Ranging from topical over-the-counter antibiotic ointments (such as the ever-popular Neosporin) to intravenously injected antibiotic solutions, these drugs have proven effectiveness in eliminating bacterial infections that arise from minor cuts and scrapes as well as life-threatening system-wide infections.

Early antibiotics were discovered and isolated from fungal molds which produced them as natural defence mechanisms against bacterial infection. More recently, newer classes of antibiotics have been created synthetically in laboratories. Because the targets of antibiotics are specific to bacterial rather than human cells, they generally have few side effects and are considered safe for the vast majority of people.

## Side Effects

While antibiotics are safe for most people, a small percentage of individuals are prone to having allergic reactions to antibiotics such as

- Resistance of organisms to antibiotics.
- Penicillin and others. Symptoms include rash, respiratory problems, low blood pressure, and swelling in the throat.
- Use of antibiotics may interfere with birth control, although these effects may not occur in all women

## Commonly used Antibiotics

Pencillins : Amoxicillin, Ampicillin, Benzylpenicillin, Phenoxymethylpenicillin. Macrolides: Clarithromycin, Erythromycin.

Cephalosporins: Cefaclor, Cefalexin, Cefataxime. Tetracyclines :Doxycyclin, Oxytetracycline, Tetracycline.

Aminoglycosides: Gentamicin, Neomycin.

Quinolones: Ciprofloxacin, Ofloxacin, Norfloxacin.

**Resistance:** Antibiotic resistance is the ability of a microorganism to withstand the effects of antibiotics. It is a specific type of drug resistance. Antibiotic resistance evolves naturally via natural selection acting upon random mutation, but it could also be engineered by applying an evolutionary stress on a population. Once such a gene is generated, bacteria can then transfer the genetic information in a horizontal fashion (between individuals) by plasmid exchange. If a bacterium carries several resistance genes, it is called multiresistant or, informally, asuperbug. The term antibacterial resistance is sometimes used to explicitly encompass organisms of bacteria.

**Reasons to develop Resistance:** The common reasons to develop antibiotic resistance in organisms are repeated using of antibiotics, differential dose, repeated infections, and environmental changes.

## Antibacterial Resistance in India:

Antibacterial resistance is a natural biological phenomenon of response of bacteria to the selective pressure of an antibiotic. In recent years, emergence of macrolide-resistant *S. pyogenes* was reported in some areas of the world. Currently, the majority (80-90%) of *Staphylococcus aureus* strains in the community is beta-lactamase producers and thus is resistant to penicillin and ampicillin. However, these strains are susceptible to beta-lactamase resistant beta-lactam antibiotics such as nafcillin, methicillin or oxacillin. Recently more than 90% *Staphylococcus aureus* isolates from South Maharashtra have been found resistant to ampicillin, tobramycin, penicillin, erythromycin, kanamycin and gentamicin; whereas, only 39.1% strains are resistant to methicillin.

## Herbal treatment:

Nature has served as a rich repository of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources, notably of plant origin. Herbal medicine, based on their traditional uses in the form of powders, liquids or mixtures, has been the basis of treatment for various ailments

in India since ancient times. emergence of multiple drug resistant strains of microorganisms due to indiscriminate use of antibiotics to treat infectious diseases has generated a renewed interest in herbal medicine Thevarious medicinal plants and their phytoconstituents used in treating bacterial infectionsare listed below

**Table:** List of medicinal plants and their phytoconstituents having Antibacterial activity

| Sl.no | Plant name                      | Phytoconstituent          |
|-------|---------------------------------|---------------------------|
| 10.   | <i>Pimenta dioica</i>           | Eugenol                   |
| 11.   | <i>Malus sylvestris</i>         | Phloretin                 |
| 12.   | <i>Withaniasomniferum</i>       | Withafarin A              |
| 13.   | <i>Berberis vulgaris</i>        | Berberine                 |
| 14.   | <i>Piper betel</i>              | Catechols, eugenol        |
| 15.   | <i>Piper nigrum</i>             | Piperine                  |
| 16.   | <i>Vaccinium spp.</i>           | Fructose                  |
| 17.   | <i>Schinus terebinthifolius</i> | Terebinthone              |
| 18.   | <i>Ranunculus bulbosus</i>      | Protoanemonin             |
| 19.   | <i>Anacardium pulsatilla</i>    | Salicylic acids           |
| 20.   | <i>Rhamnus purshiana</i>        | Tannins                   |
| 21.   | <i>Matricaria chamomilla</i>    | Anthemis acid             |
| 22.   | <i>Larrea tridentate</i>        | Nordihydroguaiaretic acid |
| 23.   | <i>Capsicum annum</i>           | Capsaicin                 |
| 24.   | <i>Syzygiumaromaticum</i>       | Eugenol                   |
| 25.   | <i>Erythroxylum coca</i>        | Cocaine                   |
| 26.   | <i>Eucalyptus globules</i>      | Tannin                    |
| 27.   | <i>Vicia faba</i>               | Fabatin                   |
| 28.   | <i>Allium sativum</i>           | Allicin, ajoene           |
| 29.   | <i>Gloriosa superb</i>          | Colchicine                |
| 30.   | <i>Centella asiatica</i>        | Asiatocoside              |
| 31.   | <i>Camellia sinensis</i>        | Catechin                  |
| 32.   | <i>Cannabis sativa</i>          | $\beta$ -Resercyclic acid |
| 33.   | <i>Lawsoniainermis</i>          | Gallic acid               |
| 34.   | <i>Humulus lupulus</i>          | Lupulone, humulone        |
| 35.   | <i>Rabdosiatrichocarpa</i>      | Trichorabdal A            |
| 36.   | <i>Lawsonia</i>                 | Lawsone                   |
| 37.   | <i>Millettiathonningii</i>      | Alpinumisoflavone         |
| 38.   | <i>Melissa officinalis</i>      | Tannins                   |
| 39.   | <i>Glycyrrhiza glabra</i>       | Glabrol                   |
| 40.   | <i>Arnica Montana</i>           | Helanins                  |

|     |                           |           |
|-----|---------------------------|-----------|
| 41. | <i>Quercus rubra</i>      | Tannins   |
| 42. | <i>Olea europaea</i>      | Hexanal   |
| 43. | <i>Allium cepa</i>        | Allicin   |
| 44. | <i>Mahonia aquifolia</i>  | Berberine |
| 45. | <i>Anemone pulsatilla</i> | Anemonins |

### Sterilization:

Sterilization is a process by which an article, surface or medium is freed of all living microorganisms either in the vegetative or spore state.

Micro-organisms are ubiquitous, since they cause contamination, infection and decay. It becomes necessary to remove or destroy them from materials or from areas. For achieving sterilization, disinfectants are using which destructs or removal of all pathogenic organisms or organisms capable of giving rise to infection. The term Antisepsis is used to indicate the prevention of infection, usually by inhibiting the growth of bacteria in wounds or tissues.

The various agents used in sterilization are two types.

### Physical agents:

Sunlight, drying, dry heat-flaming, incineration, hot air, moist heat - pasteurisation, boiling, steam under pressure, filtration candles, asbestos pods, membranes, radiation and ultrasonic vibrations.

### Chemicals agents:

**Alcohols:** Ethyl, Isopropyl, Trichlorobutanol.

**Aldehydes:** Formaldehyde, Glutaraldehyde.

Dyes, Halogens, Phenols, Surface active agents, Metallic salts

**Gases:** Ethylene oxide, Formaldehyde.

i) Dry heat sterilization: Hot air oven.

ii) Moist heat sterilization: Autoclave.

### AMOXICILLIN

#### Drug description:

Amoxicillin, a semisynthetic antibiotic, an analog of ampicillin, with a broad spectrum of bactericidal activity against many gram-positive and gram-negative microorganisms.

#### SYSTEMATIC (IUPAC) NAME:

Chemically, it is (2*S*,5*R*,6*R*)-6-[(*R*)-(-)-2-amino-2-(*p*hydroxyphenyl)acetamido]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate.

The amoxicillin molecular formula is C<sub>16</sub>H<sub>19</sub>N<sub>3</sub>O<sub>5</sub>S•3H<sub>2</sub>O, and the molecular weight is 419.45. Capsules, tablets, and powder for oral suspension of AMOXIL are intended for oral administration.

**Mode of action:** Amoxicillin acts by inhibiting the synthesis of bacterial cell walls. It inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the **cell wall** of **Gram-positive** bacteria.

**Susceptible gram positive organisms:**

- *Streptococcus* spp.
- Penicillin-susceptible *Streptococcus pneumoniae*
- Non  $\beta$ -lactamase-producing *Staphylococcus* spp.
- *Enterococcus faecalis*.

**Susceptible gram negative organisms:**

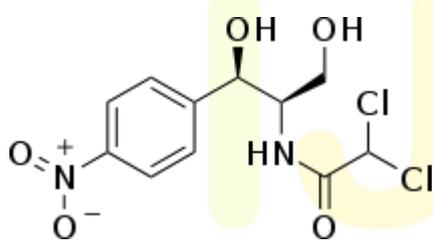
- *Haemophilus influenzae*
- *Neisseria gonorrhoeae*
- *Neisseria meningitidis*
- *Escherichia coli*

**Side effects:**

Side effects are as those for other beta-lactam antibiotics. Side effects include nausea, vomiting, and easy fatigue. Loose bowel movements (diarrhea) also may occur. The onset of an allergic reaction to amoxicillin can be very sudden and intense - emergency medical attention must be sought as quickly as possible.

**1.11 Chloramphenicol**

Chloramphenicol



**Clinical data**

|                       |   |
|-----------------------|---|
| <b>Trade names</b>    | Pentamycetin, Chloromycetin, others <sup>[71]</sup>                         |
| <b>AHFS/Drugs.com</b> | Monograph   |
| <b>MedlinePlus</b>    | a608008   |
| <b>License data</b>   | <ul style="list-style-type: none"> <li>• US FDA: Chloramphenicol</li> </ul> |

|                           |                              |
|---------------------------|------------------------------|
| <b>Pregnancy category</b> | • AU: A                      |
|                           | • US: C (Risk not ruled out) |

|                                |                                       |
|--------------------------------|---------------------------------------|
| <b>Route of administration</b> | Topical (eye drops), by mouth, IV, IM |
|--------------------------------|---------------------------------------|

|                             |                             |
|-----------------------------|-----------------------------|
| <b>Pharmacokinetic data</b> |                             |
| <b>Bioavailability</b>      | 75–90%                      |
| <b>Protein binding</b>      | 60%                         |
| <b>Metabolism</b>           | Liver                       |
| <b>Biological half-life</b> | 1.6-3.3 hours               |
| <b>Excretion</b>            | Kidney (5-15%), faeces (4%) |

#### Identifiers

IUPAC name[show]

**CAS Number** • 56-75-7

#### Chemical and physical data

**Formula**  $C_{11}H_{12}Cl_2N_2O_5$

**Molar mass** 323.1320 g/mol

**Chloramphenicol** is an antibiotic useful for the treatment of a number of bacterial infections. This includes as an eye ointment to treat conjunctivitis. By mouth or by injection into a vein it is used to treat meningitis, plague, cholera, and typhoid fever. Its use by mouth or by injection is only recommended when safer antibiotics cannot be used and if used monitoring both blood levels of the medication and blood cell levels every two days is recommended during treatment.

#### Medical uses

The original indication of chloramphenicol was in the treatment of typhoid, but the now almost universal presence of multiple drug-resistant *Salmonella typhi* has meant it is seldom used for this indication except when the organism is known to be sensitive. Chloramphenicol may be used as a second-line agent in the treatment of tetracycline-resistant cholera.

#### Adverse effects

- Aplastic anemia
- Bone marrow suppression
- Leukemia

- Gray baby syndrome
- Hypersensitivity reactions

## **INDIAN ORIGIN MEDICINAL PLANTS:**

### **NEEM**



**SCIENTIFIC NAME:** Azadirachta indica

**TELUGU NAME:** Vepa

**SYNONYM:** Margosa

**BIOLOGICAL SOURCE:** Azadirachta indica

**FAMILY:** Meliaceae

**CHEMICAL CONSTITUENTS:** azadirachtin and the others are nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin, and quercetin.

**DESCRIPTION:** The plant product or natural products show an important role in diseases prevention and treatment through the enhancement of antioxidant activity, inhibition of bacterial growth, and modulation of genetic pathways. The therapeutics role of number of plants in diseases management is still being enthusiastically researched due to their less side effect and affordable properties. It has been accepted that drugs based on allopathy are expensive and also exhibit toxic effect on normal tissues and on various biological activities.

**MEDICINAL USES:** immunomodulatory, anti-inflammatory, antihyperglycaemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and anticarcinogenic properties.

### **Antibacterial activity**

Recent research shows the isolation and identification of the antibacterial active compound from petroleum ether extract of neem oil. The study of Zhong *et al.* showed an antibacterial activity of 9-octadecanoic acid-hexadecanoic acid-tetrahydrofuran-3,4-diyl ester from neem oil. Elavarasu *et al.* studied *in vitro* anti-plaque microbial activity of neem oil.

### **Antiviral**

Galhardi *et al.* studied the *in vitro* antiviral property of *Azadirachta indica* polysaccharides for poliovirus. The study of Saha *et al.* showed water extracted polysaccharides from *A. indica* leaves with anti-bovine herpes virus type 1 (BoHV-1) activity. The research of Xu *et al.* showed the *in vitro* antiviral activity of neem seed kernel extracts against duck plague virus. Tiwari *et al.* showed the *in vitro* antiviral activity of neem (*A. indica* L.) bark extract against herpes simplex virus type-1 infection.

### **Sexually transmitted disease**

Few researchers have focused on neem efficacy in treating sexually transmitted diseases. The reports that have been completed are overwhelmingly positive. Recent research of Shokeen *et al.* showed the evaluation of the activity of 16 medicinal plants against *Neisseria gonorrhoeae*.

### **Neem and the immune system**

Thoh *et al.* studied that azadirachtin interacts with the tumor necrosis factor (TNF) binding domain of its receptors and inhibits TNF induced biological responses.

### **Anti-inflammatory activity**

The study of Alam *et al.* showed the anti-inflammatory activity of epoxyazadiradione against macrophage migration inhibitory factor. Thoh *et al.* found that azadirachtin interacts with retinoic acid receptors and inhibits retinoic acid-mediated biological responses.

### **Antioxidant effect**

Manikandan *et al.* researched that antioxidant and protective effects of active neem leaf fractions against hydrogen peroxide induced oxidative damage to pBR322 DNA and red bloodcells.

## Anticarcinogenic activity

Chatterjee *et al.* showed that identification of a sulfonoquinovosyldiacylglyceride from *A. indica* and studies on its cytotoxic activity and DNA binding properties. Perumal *et al.* studied ethanolic neem (*A. indica* A. Juss) leaf extract induced apoptosis and inhibits the IGF signaling pathway in breast cancer cell lines. Aravindan *et al.* showed that molecular basis of 'hypoxic' breast cancer cell radio-sensitization with phytochemicals. Induction of apoptosis in human breast cancer cells by nimbolide were carried out by Elumalai *et al.* Srivastava *et al.* showed that neem oil limonoids induces p53-independent apoptosis and autophagy. A review of the anticancer biology of *Azadirachta indica* was carried out by Paul *et al.* Research of Veeraraghavan *et al.* showed the effect of neem leaf extract on rel protein-regulated cell death/radiosensitization in pancreatic cancer cells. Mahapatra *et al.* showed novel molecular targets of *Azadirachta indica* associated with inhibition of tumor growth in prostate cancer .

## Skin diseases

Neem has a remarkable effect on chronic skin conditions. Acne, psoriasis, eczema, ringworm and even stubborn warts are among the conditions that can clear up easily when high quality, organic neem oil is used. Neem oil and leaves has been used in Siddha medicine for the treatment of skin diseases. In addition, neem oil can be used as an excellent component of cosmetics to help clear, beautify and rejuvenate the skin.

## Digestive disorders

Neem is generally accepted in the ayurvedic medical tradition as a therapy for ulcers and other types of gastric discomfort. Neem promotes a healthy digestive system by protecting the stomach, aiding in elimination and removing toxins and harmful bacteria. Bandyopadhyay *et al.* studied the neem bark extract of gastroprotective effect.

## Parasitic diseases

Historically, neem has been used to rid the body of all forms of parasites. Neem quickly kills external and internal parasites. Neem extracts have hormone mimics that interfere with the lifecycle of parasites, inhibit their ability to feed and prevent the eggs from hatching. Abdel *et al.* studied the efficacy of a single treatment of head lice with a neem seed extract. Luong *et al.* found that neem leaf slurry is a sustainable, natural product and anopheline larvicide in west African Villages.

## ALOE VERA



**SCIENTIFIC NAME:** Aloe barbadensis miller.

**TELUGU NAME:** aloe

**SYNONYM:** Aloe

**BIOLOGICAL SOURCE:** Aloe vera

**FAMILY:** Liliaceae

**CHEMICAL CONSTITUENTS:** Aloe vera plant extract are chromone and anthraquinone and its glycoside derivatives, alongside others such as phenylpyrone derivatives, flavonoids, phenylpropanoids, coumarins, phytosterols, naphthalene analogs, lipids, and vitamins.

### Description

Traditional medicine is in practice for many centuries by a substantial proportion of the population in many countries. It is recognized that in some developing countries, plants are the main medicinal source to treat various infectious diseases. Plant extracts represent a continuous effort. The name is derived from the Arabic word 'alloeh' which means 'bitter', referring to the taste of the liquid contained in the leaves. Aloe that is believed to have originated in the Sudan. *Aloe vera* grows in arid climates and is widely distributed in Africa, India and other arid areas. The species is frequently cited as being used in herbal medicine. *Aloe vera* is a perennial, drought resisting, succulent plant. It has stiff green, lance-shaped leaves containing clear gel in a central mucilaginous pulp. Aloe gel can help to stimulate the body's immune system (Davis, 1997). The use of plant product for pharmaceutical purpose has been gradually increased.

## Antibacterial activity

The antibacterial studies were carried out by disc diffusion technique. The sterile nutrient agar plates and potato dextrose agar plates were prepared. The bacterial test organisms like *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Escherichia coli* were spread over the nutrient agar plates using separate sterile cotton buds.

After the microbial lawn preparation, three different extracts (20 grams of powdered plant materials mixed with 100 ml of various solvents (distilled water, ethanol, and acetone solution)) of plant disc were placed on the organism-inoculated plates with equal distance; control discs were also prepared. All bacterial plates were incubated at 27°C for 24 h. The diameter of the minimum zone of inhibition was measured in millimeter. For each test, three replicates were performed.

## Antioxidant activity

The antioxidant activities of the extracts were determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The DPPH radical scavenging activity test was determined following Cheung *et al.* and Shang *et al.* The reduction of DPPH radicals was estimated by measuring the absorption at 517 nm. The percentage of DPPH scavenging activity (AA%), was calculated using the equation:  $AA\% = 100 [(A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}}]$ , where  $A_{\text{control}}$  is the initial absorbance of the methanolic DPPH solution, and  $A_{\text{sample}}$  is the reaction mixture at 515 nm (DPPH + sample).

## antiviral activity

Anthraquinone derivatives like aloe-emodin, emodin and chrysophanol, reportedly exhibit antiviral activity. Previous findings<sup>46</sup> have recorded the inhibitory effect of 0.2–5% Aloe vera gel (extracted in 2% dimethyl sulfoxide (DMSO)) on herpes simplex virus in Vero cell line. AMLA



**SCIENTIFIC NAME:** Phyllanthus emblica

**TELUGU NAME:** Amla

**SYNONYM:** Emblica officinalis

**BIOLOGICAL SOURCE:** fruits

**FAMILY:** Phyllanthaceae.

**CHEMICAL CONSTITUENTS:** vitamin C (ascorbic acid) and contains several bioactive phytochemicals, of which majority are of polyphenols like ellagic acid, chebulinic acid, gallic acid, chebulagic acid, apigenin, quercetin, corilagin, leucolin.

## Description

*Emblica officinalis* Gaertn or *Phyllanthus emblica* Linn, belonging to the family Euphorbiaceae, is a plant originally native to India but is today also found growing in Pakistan, Uzbekistan, Sri Lanka, Southeast Asia, China, and Malaysia.<sup>1</sup> In colloquial terms, they are known as Indian gooseberry tree and emblic myrobalans, Malacca tree in English, and amla in Hindi. The fruits are yellowish green in color, globular in shape, fleshy, and smooth striated with an obovate obtusely triangular six-celled nut. The fruits are of culinary use and are widely used to make pickles, chutneys, and as a vegetable in various dishes. They are also used to prepare a sweet delicacy by name murabbah, where the ripe fruits are soaked in concentrated sugar syrup for extended period till the aroma of the fruits exudates into the sugar syrup. The ripe fruits are also used to prepare fresh juice and are useful during summer.

## Antimicrobial activity

The crude extract of seed was tested for antibacterial and antifungal activity. Drugs like gentamycin (10µg) and DMSO used as control. Antibacterial activity of crude samples in different solvents were tested by disc diffusion technique against pathogenic organisms such as *E. Coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsella pneumonia*. The nutrient agar plates were inoculated with 0.1 ml of pathogenic microbes by spread plate method. The Whatmann filter paper disc were sterilized and inoculated with the samples and DMSO was kept as negative control. All the plates were incubated at 30°C for 24 hours to measure the zone of inhibition.

## Antibacterial activity

The antibacterial action of EO is higher for Gram-positive bacteria, while its effectiveness is limited for countering fungi. The extracts of EO exhibited high zone of inhibition (ZOI) when tested for *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae* and *Candida albicans*. Antimicrobial effectiveness for Gram-positive, Gram-negative bacteria along with fungal agents reflects usage of fruit of *P. emblica* as a remedy for different microbial diseases.

## Antifungal activity

The *P. emblica* extract analyzed for *in vitro* potential against *Fusarium solani*, a fungal agent causing dry potato tuber rot revealed inhibition of mycelial growth at a 100% concentration.

The aqueous extract of EO reported significant antifungal activity against eight species of *Aspergillus* fungi

(*Aspergillus candidus*, *A. columnaris*, *A. flavipes*, *A. flavus*, *fumigatus*, *A. niger*, *A. ochraceus*, and *A. tamari*). In this study, different solvents employed for the extraction process included Petroleum ether, Chloroform, Methanol, Benzene, and Ethanol. Plant methanolic extract of EO was not having antifungal activity for phytopathogenic fungus *A. niger* F2723. The aqueous extracts of EO revealed a diverse degree of antimicrobial action for the pathogenic microbes viz., *S. aureus*, *E. coli* and *Candida* species.

### **Antiviral activity**

*P. emblica* contains different class of secondary metabolites. Phyllaemblicin B extracted from plant roots showed inhibitory potential for Coxsackie virus while phenolic content revealed effectiveness for herpes simplex viruses (HSV) 1 and 2. 1, 2, 4, 6-tetra-O-galloyl- $\beta$ -D-glucose from *P. emblica* showed antiviral activity for HSV *in vitro*.

Pentagalloylglucose inhibits influenza A virus replication by prevention of adsorption of the virus and suppressing release of virus.

Several plant extracts possess potential to act against HIV via inhibition of viral enzymes. *P. emblica* plant extracts may have anti-HIV property by inhibiting reverse transcriptase enzyme of the virus.

**Immunomodulatory Effect:** Laboratory experiment in mice showed that an aqueous *P. emblica* extract natural killer cell activity and antibody-dependent cellular cytotoxicity in mice

**Antitussive Effect:** The antitussive activity of *E. officinalis* was tested in conscious cats by mechanical stimulation of the laryngopharyngeal and tracheobronchial mucous areas of airways. The ethanol extract of the fruits of *E. officinalis* seems to have a good ability to inhibit mechanically-provoked cough.

### **CLOVE**



**SCIENTIFIC NAME:** *Syzygium aromaticum*

**TELUGU NAME:** Lavangaalu

**SYNONYM:** Clove

**BIOLOGICAL SOURCE:** Dried flower buds of plant

**FAMILY:** Myrtaceae

**CHEMICAL CONSTITUENTS:** At least 30 compounds have been identified eugenol is the major compound, accounting for at least 50%. The remaining 10–40% is made up of eugenyl acetate,  $\beta$ -caryophyllene, and  $\alpha$ -humulene. Less than 10% correspond to minor or trace components such as diethyl phthalate, caryophyllene oxide, cadinene,  $\alpha$ -copaene, 4-(2-propenyl)-phenol, chavicol, and  $\alpha$ -cubebene.

## Description

*Syzygium aromaticum* L. belong to the *Myrtaceae* family, which has more than 3000 species and 130–150 genera, such as the myrtle, eucalyptus, clove, and guava families. Clove is an aromatic flower cultivated in Madagascar, Sri Lanka, Indonesia, and China. Several reports suggest that *S. aromaticum* L. contains approximately 15–20% wt. of EO. CEO contains a high amount of phenolic compounds with several biological activities, including antibacterial, antifungal, insecticidal, and antioxidant properties. The FDA classifies CEO as generally recognized as safe (GRAS); for this reason, it is used in perfumes, cosmetics, sanitary products, medicines, and foods.

## Antimicrobial

The antibacterial mechanism has been related to the -OH groups located at the meta and ortho positions, respectively, in the main chemical composition. These functional groups can interact with the cytoplasmic membrane of microbial cells. CEO can permeate through the cell membrane due to its lipophilic properties. The interaction of CEO with polysaccharides, fatty acids, and phospholipids causes loss of cellular membrane integrity, leakage of cellular contents, and interference with proton pump activity, leading to cell death. CEO can inhibit Gram-negative bacteria (*E. coli*, *Salmonella*, *Klebsiella pneumoniae*, *Erwinia carotovora*, *Agrobacterium*, and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*S. aureus*, *Streptococcus*, and *L. monocytogenes*), *Aspergillus* (*A. flavus*, *A. parasiticus*, and *A. ochraceus*), *Penicillium*, *C. albicans*, and yeast.

## Antioxidant

It has the antioxidant compounds eugenol, eugenyl acetate,  $\beta$ -caryophyllene, and  $\alpha$ -humulene, which protect cells from free radical oxidation. Diseases such as cancer, arteriosclerosis, Alzheimer's disease, and Parkinson's disease are related to the presence of ROS compounds. CEO has shown scavenging activity on radicals and inhibition of lipid peroxidation. The hydroxyl group available in eugenol on the aromatic ring is responsible for the antioxidant activity. The phenolic compounds transfer electrons or hydrogen atoms and neutralize them to free radicals, resulting in a blocked oxidative process.

## Antiviral

CEO has shown antiviral activity against Ebola, influenza A virus, and herpes simplex virus types 1 and 2. Recent studies by de Oliveira et al. showed that eugenol derivatives could inhibit the activity of the West Nile Virus, providing a promising compound against flaviviruses such as dengue, Zika, and yellow fever. Eugenol has also

been studied as a possible inhibitor of the initial stage of HIV-1 infection because it can reduce virus replication. Likewise, eugenol can increase lymphocyte production; therefore, the lymphocyte proliferation capacity of eugenol may be responsible for its anti-HIV-1 activity. It has demonstrated antiviral activity against feline calicivirus, which is used as a substitute for human norovirus.

### **Anti-Inflammatory and Wound Healing**

Oxidative stress and inflammation are near-related processes in many pathophysiological conditions such as diabetes, hypertension, and cardiovascular and neurodegenerative diseases

. The anti-inflammatory properties of CEO and eugenol are comparable to diclofenac gel, reducing inflammation by 60 to 20% after 3 h. Likewise, induced wounds in rats treated with CEO showed a significant contraction of more than 95% in the first 15 days. These results demonstrate that animals treated with CEO underwent similar healing to those treated with neomycin, which is currently used to control inflammation and heal wounds. Therefore, the chronic and acute side effects of synthetic antibiotics can be avoided.

### **TEA**



**SCIENTIFIC NAME:** *Camellia sinensis*

**TELUGU NAME:** Chai

**SYNONYM:** Decoction.

**BIOLOGICAL SOURCE:** Leaves and leaf buds

**FAMILY:** Theaceae

**CHEMICAL CONSTITUENTS:** The leaves of tea consist of thease which is an enzymatic mixture containing an oxidase, which partly converts the phlobatannin into phlobaphene, as chemical constituent.

- Other chemical constituent present in tea leaves are tannins, caffeine.
- It contain 1-5% of tannin and 10-24% of caffeine. In tea leaves theobromine is also present in small amount. Tea leaves also consist of theophylline and volatile oil. Alkaloid content also present in tea leaves but its amount only depends on season and age of tea leaves.

- Physically, tea has both qualities of solution and suspension. Caffeine is about 3% of tea's dryweight. Black tea contains dietary mineral manganese about 0.5 milligram. Fluoride is also present in tea in small amount. Polyphenols are most abundant chemical constituents present in tea. (30-40%).

## Antifungal Activity

Wang et al. tested the inhibitive effects of different TP concentrations on three species of plant pathogenic fungi, *Bipolaris maydis*, *Colletotrichum musae* and *Fusarium oxysporum*. The results showed that TP significantly inhibited hyphal growth and spore germination of the three fungi, and the inhibitive effects were directly proportional to the concentration of TP solutions.

## Antibacterial Activity

In addition to antifungal activity, TP showed inhibitory effect on various phytopathogenic bacterial infections. Fukai et al. reported the antibacterial activity of TP measured as minimum inhibitory concentration (MIC) against phytopathogenic bacteria, including eight strains of *Erwinia*, 10 strains of *Pseudomonas*, and one strain each of *Clavibacter*, *Xanthomonas* and *Agrobacterium*. These bacteria tend to infect commonly cultivated vegetables such as lettuce, tomatoes, eggplants, cabbage, radish, Irish potatoes, onions, and grapes. After three days incubation of the bacterial agar plates containing different concentrations of individual TPs, EGC and EGCG showed more inhibitory effect than EC and ECG against the test bacteria, and MICs were mostly below 100 ppm.

## Antiviral Activity

Having noticed the antiviral effect of tea infusion on tobacco mosaic virus (TMV), Okada and Furuya tested the inhibitory effect of each TP component and its own mix against TMV and cucumber mosaic virus (CMV) on tobacco leaves. The aqueous solutions of TPs were injected into the soil around the base of the plants systemically infected with TMV and CMV.

## COMPARATIVE STUDIES OF ANTIMICROBIAL ACTIVITIES OF NEEM, AMLA, ALOE, ASSAM TEA AND CLOVE EXTRACTS BASED ON Mehrotra et al 2010.

### DESCRIPTION:

This comparative study is a review of an article by Mehrotra et al., 2010, following the title *Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against Vibrio cholerae, Staphylococcus aureus and Pseudomonas aeruginosa* by Shubhi Mehrotra, Ashwani K. Srivastava and Shoma Paul Nandi.

### MATERIALS AND METHODS

Collection and pre-extraction of plant materials Neem and Aloe leaves were collected from the Amity Institute of Organic Agriculture Farm, Noida, UP, India. Amla fruits were collected from Bharmar village, district Kangra in Himachal Pradesh. Assam Tea leaves were collected from a local tea farm in Assam, India. Clove buds were purchased from a local market in Noida, UP, India.

## PREPARATION OF PLANT EXTRACTS

Ethanol extracts were prepared as described previously (Ghoshal et al., 1996) with the following modifications. Ten grams of the plant materials were pounded manually with mortar and pestle and soaked in 40 ml absolute ethanol in 250 ml sterile conical flasks incubated at 37 °C incubator with shaking at 120 rpm for 24 h.

## PATHOGENS

*V. cholerae* strain was obtained from National Institute of Cholera and Enteric Diseases, Kolkata, India. Methicillin resistant, *S. aureus* and *P. aeruginosa* were procured from Nu Life Consultants and Distributors Pvt. Ltd., Lajpat Nagar, New Delhi. Strains of bacteria were maintained at 4°C on LB plates and were sub-cultured (24 h, 37 °C) prior to use. Purity of the cultures was checked at regular intervals as described by Acheampong et al. (1988).

## DETERMINATION OF ANTIMICROBIAL ACTIVITY OF EXTRACTS

Standardized inoculum (100 µl) of 0.5 McFarland turbidity standard, that is, equivalent to  $5 \times 10^8$  cfu/ml (Lopez-Brea et al., 2008) of each test bacterial strains was spread using a sterile glass spreader onto sterile LB solid media plates so as to achieve even growth. The plates were allowed to dry and then a sterile cork borer (8.0 mm diameter) was used to bore wells in the agar plates. The extracts (50 µl/well) were loaded in the wells and absolute ethanol (50 µl/well) was taken as negative control. The plates were then incubated at 37°C for 24 h.

Antimicrobial activity of the extracts was determined by measuring the diameter of inhibition zone in milli-meter produced against the pathogens. The experiment was done three times and the mean values were calculated. To determine the minimum inhibitory concentration (MIC), serial dilutions of the extracts were done and assayed by agar well diffusion. The extracts were made out of 10 g dry weight sample and dissolved in the final volume of 5 ml ethanol leading to the concentration of plant extract as 2 µg/µl.

## ANTIBIOTIC DISC ASSAY

The plates were prepared as mentioned above. The antibiotic discs of tetracycline, ampicillin, vancomycin and kanamycin each of 7.0 mm diameter (Hi-media) were placed using sterile forceps on the agar plates. The plates were then incubated at 37°C for 24 h. Susceptibility of the antibiotics against the test strains was determined by measuring the diameter of zone of inhibition (mm) produced against the test strains. The experiment was performed three times and the mean values were calculated.

## TLC SEPARATION, CONTACT BIOAUTOGRAPHY AND PH STABILITY

Plant extracts were separated using pre-coated silica plates (Merck 60F-254) and running buffer composition as mentioned with the results. The TLC plates were cut into thin strips and placed with silica side down on the bacterial plate and growth inhibition was monitored. For pH stability assay, the TLC strips were treated with 100 mM of citrate buffer (100 µl) having pH 2.0, pH 7.0 and pH 8.0 separately for 1 h, after which bioautography was performed. R<sub>f</sub> values were measured as the ratio of mobility for bioactive zone to the total length of the run.

## RESULTS AND DISCUSSION

### Antibiotic activity of plant extracts

In the present study, we identified five plants such as *Azadirachta indica* (Neem), *Aloe vera* (Aloe), *Embllica officinalis* (Amla), *Camellia sinensis assamica* (Assam tea) and *Syzygium aromaticum* (Clove) that are effective against all the three target pathogens *S. aureus*, *V. cholerae* and *P. aeruginosa*. Ethanolic extracts of these plants were serially diluted and the MIC values were determined (Table 1). As shown, all these five plants have the potential to control the growth of all the three pathogens. Extracts of Amla pulp and Clove buds were found to be highly efficient in controlling the growth of all tested pathogens with MIC values of  $0.025 \mu\text{g}/\mu\text{l}$ ; whereas, MIC of Neem, Aloe and Assam tea extracts ranged from 0.1 to  $0.5 \mu\text{g}/\mu\text{l}$  (Table 1). In terms of sensitivity against standard antibiotics, as shown in Table 1, *S. aureus* and *V. cholerae* strains were resistant to 30  $\mu\text{g}$  of ampicillin, but were sensitive to 30  $\mu\text{g}$  of kanamycin, vancomycin and tetracycline. The *P. aeruginosa* pathogen showed sensitive response to all of the antibiotics tested.

| Plant extracts                   | Pseudo    | SMR       | Vibrio    |
|----------------------------------|-----------|-----------|-----------|
| Neem                             | 0.25      | 0.1       | 0.3       |
| <i>Aloe vera</i>                 | 0.35      | 0.1       | 0.3       |
| Amla pulp                        | 0.025     | 0.025     | 0.025     |
| Assam tea                        | 0.5       | 0.1       | 0.25      |
| Clove bud                        | 0.025     | 0.025     | 0.025     |
| Kanamycin (30 $\mu\text{g}$ )    | Sensitive | Sensitive | Sensitive |
| Vancomycin (30 $\mu\text{g}$ )   | Sensitive | Sensitive | Sensitive |
| Tetracycline (30 $\mu\text{g}$ ) | Sensitive | Sensitive | Sensitive |
| Ampicillin (30 $\mu\text{g}$ )   | Resistant | Resistant | Resistant |

**Table:** MIC OF PLANT EXTRACTS OBTAINED BY AGAR CUP DIFFUSION ASSAY

**Table 2.** Stability of plant extracts at different temperatures.

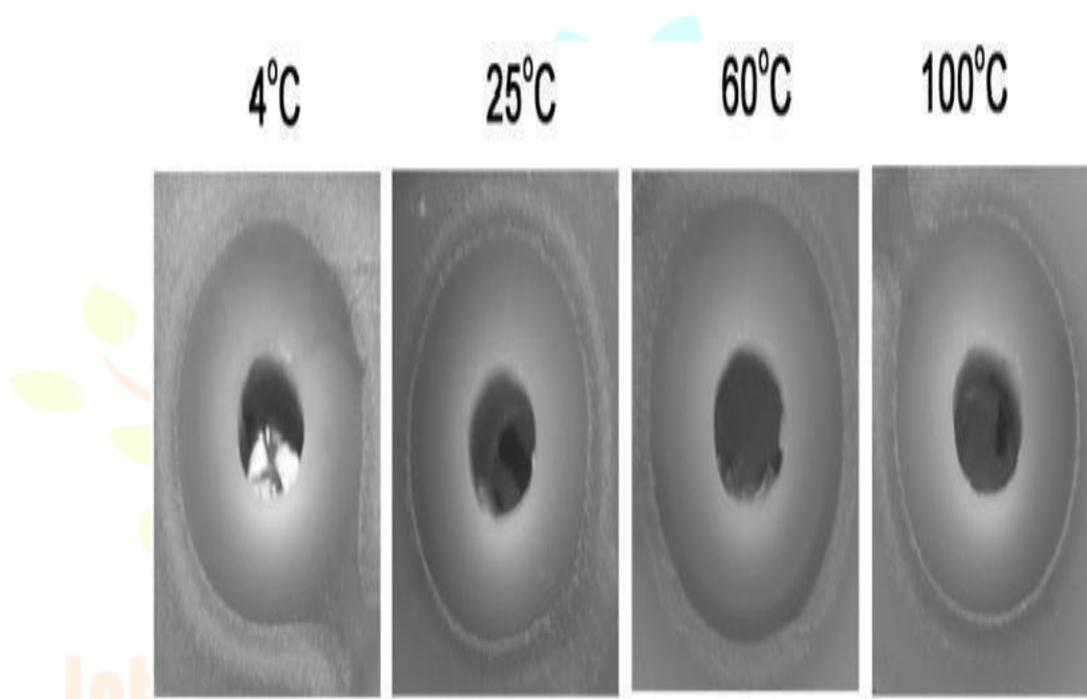
| Plant extract    | Neem      |    |    |     | Aloe  |    |    |     | Amla |    |    |     |
|------------------|-----------|----|----|-----|-------|----|----|-----|------|----|----|-----|
|                  | 4         | 25 | 60 | 100 | 4     | 25 | 60 | 100 | 4    | 25 | 60 | 100 |
| Temperature (°C) | 4         | 25 | 60 | 100 | 4     | 25 | 60 | 100 | 4    | 25 | 60 | 100 |
| Pseudo           | 15        | 15 | 15 | 15  | 16    | 13 | 16 | 15  | 17   | 17 | 16 | 16  |
| SMR              | 24        | 24 | 23 | 24  | 14    | 14 | 16 | 14  | 30   | 31 | 29 | 29  |
| Vibrio           | 15        | 15 | 15 | 15  | 14    | 14 | 15 | 15  | 22   | 21 | 21 | 20  |
| Plant Extract    | Assam tea |    |    |     | Clove |    |    |     |      |    |    |     |
| Temperature (°C) | 4         | 25 | 60 | 100 | 4     | 25 | 60 | 100 |      |    |    |     |
| Pseudo           | 19        | 18 | 17 | 17  | 13    | 13 | 12 | 12  |      |    |    |     |
| SMR              | 20        | 15 | 15 | 15  | 28    | 27 | 26 | 27  |      |    |    |     |
| Vibrio           | 15        | 15 | 15 | 15  | 23    | 24 | 26 | 24  |      |    |    |     |

**Table legend:** Heat stability of plant extracts was determined by treating the plants extracts for one hour in the indicated temperature followed by measuring zone of inhibition by agar cup diffusion assay. Pseudo -*P. aeruginosa*, SMR – *S. aureus*, Vibrio – *V. cholerae*.

## Extreme temperature stability of plant extracts

The plant extracts were placed in a thermal cycler at 4, 25, 60 and 100°C temperature for oneh and antibiotic assay was performed by agar well diffusion. The zone of inhibition with 50 µl extracts (Table 2) indicates that the bioactive components were very stable over the wide

range of temperatures. The experiment was repeated three times with similar results and a representative oneis taken for generating Table 2. Zone of inhibition of *V. cholerae* with Amlaextract treated at various temperatures is shown in Figure 1.

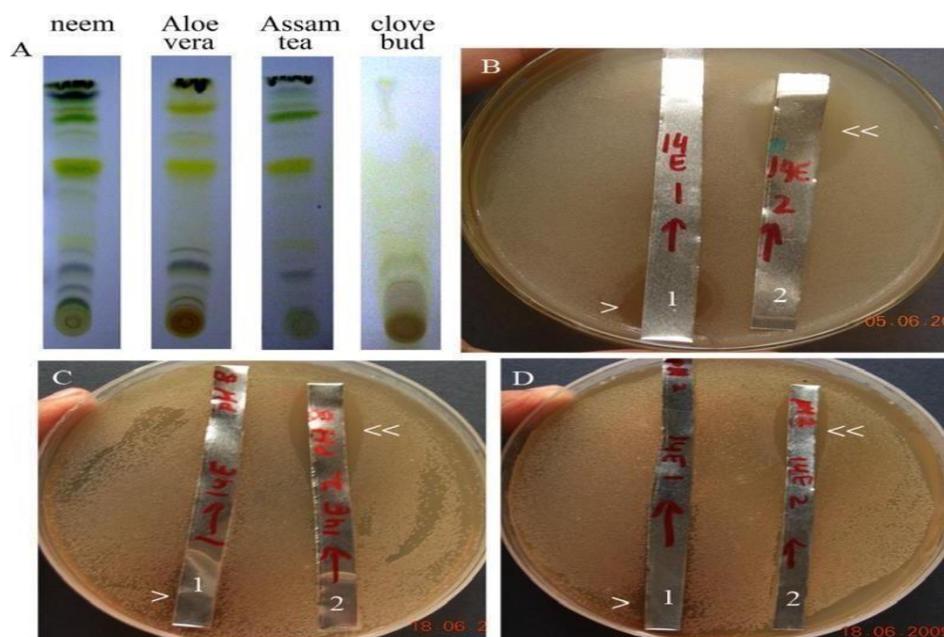


**Figure 1.** Zone of inhibition of *V. cholerae* with Amla extract after different temperature treatment. Amla extracts were treated atindicated temperature in a thermal cycler for one hour before placing the extract in agar well. Photograph was taken after.(Mehrotra et al.,2010)

## Stability of TLC separated components in different pH range

By keeping in mind the potential use of these plant extracts as oral consumption to treat pathogen infection, stability of the plant extracts were studied in acidic and alkaline pH ranges. TLC strips were drenched with citrate buffer solution of pH 2.0, 7.0 and 8.0, respectively and incubated for one hour before testing the antibiotic activity assay through contact autobiography. It was observed that the slower moving zone of inhibition was reduced after treatment with pH 2.0 and 8.0 buffers, whereas the faster moving bioactive component retainedactivity (Figures 2C and D). This study identified several novel roles of plant extracts and efficacies in controlling the growth of very challenging human pathogens. For example, Aloe vera is being used for decades as a medicinal plant (Lorenzetti et al., 1964; t Hart et al., 1990)against bacterial infection, but to our knowledge, this is the first ever report of its efficacy in controlling growth of *V. cholerae*. Similarly, the role of Amla extract in *V. cholerae* is also a novel finding of this study. However, the most important finding of this study is the identification of

bioactive components present in multiple plants having similar mobility in TLC indicating the similar kind of component to be effective against multiple pathogens.



**Figure 2.** TLC separation of plant extracts and contact autobiography. (A) TLC strips of indicated plant extracts. Photographs were taken without staining. The bands are of natural pigments present. (B) Antibiotic zones detected from TLC strip of Clove bud against *S.aureus*. (C and D) pH stability study after treating the strips with buffers of pH 8.0. (Mehrotra et al.,2010)

## Conclusion

In this I concluded that Indian origin plants have medicinal properties along with daily traditional uses and extracts of Neem, Amla, Aloe, Assam tea and Clove showed that they are effective against all the tested human pathogens *P. aruginosa*, *S. aereus*, and *V. cholerae* in controlling their growth in vitro in culture condition from (Mehrotra et al.,2010). Amla (*Emblica officinalis*) fruit, Neem (*Azadirachta indica*) leaves, Aloe (*Aloe vera*) leaves, Assam Tea (*Camellia sinensis assamica*) leaves and Clove (*Syzygium aromaticum*) buds were found to inhibit the growth of different microbial organisms. These also play a role in our daily life and are also used in many ayurvedic medicine preparations.

## ABBREVIATIONS:

**TLC**-THIN LAYER CHROMATOGRAPHY **WHO**-WORLD HEALTH ORGANISATION **TIM**-

TRADITIONAL INDIAN MEDICINE **TCM**-TRADITIONAL CHINESE MEDICINE

**MRSA**-METHICILLIN RESISTANT STEPHYLOCOCUS AUREUS

**E.COLI**-ESCHERCHIA COLI

**STD**-SEXUALLY TRANSMITTED DISEASE

**TNF**-TUMOR NECROSIS FACTOR

**DPPH**-1,1-DIPHENYL2-PICRYLHYDROXYLEO-ESSENTIAL OIL

**GRAS**-GENERALLY RECOGNISED AS SAFE **MIC**-MINIMUM INHIBITORY CONCENTRATION **EGC**-

EXPERIMENT GROUND COMPUTE

**CMV**-CUMUBER MOSAIC VIRUS

**TMV**-TOBACCO MOSAIC VIRUS

**EGCG**-EPI-GALLOCATECHIN 3-GALLATE

**RF**-RETENTION FACTOR

**TCA**-TRICHOLOROACETICACID

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