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## Research Article

### IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY OF CRUDE EXTRACTS OF MEDICINAL PLANTS AGAINST MDR PATHOGEN

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## Abstract

Wounds are perhaps in escapable events in the life of a human and at times, they are dangerous or even life threatening complication wounds are commonly infects *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*. Among these *Streptococcus pyogenes* shows highest predominance. This work is mainly focused to know the antimicrobial activity of *Tridax procumbens*, *Leucas aspera*, and *Ficus glomerata* against wound pathogens. Totally 25 wound samples were collected from Government hospital, Vandhavasi. The infectious organisms *Streptococcus pyogenes* were isolated from pus samples and identified by making use of selecting and differential media, biochemical tests in addition to microscopic methods. Herbal medicines have been used for the treatment of infectious disease. The plant chosen for the screening activity was *Tridax procumbens*, *Leucas aspera*, *Ficus glomerata*. Water, alcohol, hexane, chloroform have the antimicrobial activity. MBC of plant extract was done by using disc diffusion method. Phytochemical analysis and Thin layer chromatography (TLC) was done for all extracts. The herbal medicine better care to human mind as well as disease due to *Streptococcus pyogenes*.

**Keywords:** Wounds, *Staphylococcus aureus*, biochemical tests, antimicrobial activity

## Introduction

Skin is the first line defence mechanisms of the body. Skin is the largest and well known organ of the body, which comprises 15% of our body weight. Skin harbours large number of normal flora and has good resistant mechanism. During unfavorable condition pathogen and opportunistic pathogen will cause severe infection in the skin. Wound is defined simply as the disruption of cellular and anatomic continuity of a tissue (Bennet, 1988). It may be produced by physical, chemical, thermal, microbial or immunological insult to the tissue. It result in damage and is typically associated with loss of function. Wound are classified as acute wound which includes Surgical wound, Penetrating wound, Avulsion injury, crushing or shearing injury, Burn injury and bite

wound. Chronic wound which includes Arterial ulcer (peripheral vascular disease), venous ulcer (venous insufficiency), Lymphedema, Pressure, ulcer (Decubitus ulcer) and neuropathic ulcer (Diabetes mellitus). Wound healing consists of integrated cellular and biochemical events leading to re-establishment of structural and functional integrity with regain of strength of injured tissue. The aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences (Myers et al, 1980).

Various growth factors, macrophage derived growth factor monocyte derived growth factor (Mather et al, 1989) etc are necessary for the initiation and promotion

of wound healing. The effect of specific types of microorganisms on wound healing has been widely published, and although the majority of wounds are polymicrobial, involving both aerobes and anaerobes, aerobic pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa* and Beta hemolytic *Streptococci* have been most frequently cited as the cause of delayed wound healing and infection.

*Streptococcus pyogenes* is a gram positive, non motile, nonspore forming cocci that occurs in chains or in pairs of cells. It is one of the most frequent pathogens of humans. It was demonstrated in cases of erysipelas and wound infections by Billiroth in 1874 and in the blood of a patient with puerperal sepsis by Pasteur in 1879, Fehkisen in 1883, and isolated chain forming organisms in pure culture from erysipelas lesions and then demonstrated that these organisms could induce typical erysipelas in humans. Rosenbach applied the designation *Streptococcus pyogenes* to these organisms in 1884. Initial progress toward a rational classification of streptococci dates from the description by Schotmuller in 1903 of the blood agar technique for differentiating hemolytic from non-hemolytic streptococci. In 1919 J.H. Brown made a systemic study of patterns of hemolysis and introduced the terms alpha, beta, and gamma hemolysis.

Group A *Streptococci* (*Streptococcus pyogenes*) is one of the most important bacterial pathogens of human. This ubiquitous organism is the most frequent bacterial cause of acute pharyngitis and it also gives rise to a variety of cutaneous and systemic infections. Acute *Streptococcus pyogenes* infections may present as pharyngitis, scarlet fever, impetigo, invasive, toxigenic infections can result in necrotizing fasciitis, myositis and streptococcal toxic shock syndrome. Patients may also develop immune mediated post streptococcal sequelae, such as acute rheumatic fever and acute glomerulonephritis.

In the present study focus the control of infectious wound disease by herbal medicines such as *Tridax procumbens*, *Leucas aspera* and *Ficus glomerata*. *Tridax procumbens* Linn have been reported to have various pharmacological effect, antimicrobial activity against both Gram positive and Gram negative bacteria and stimulate wound healing (*Udopa et al*, *Diwan et al*, 1982 and *Diwan et al.*, 1983). Leaf juice is used by villages to arrest bleeding from cuts and bruises in animals. This juice accelerates two phases of healing namely epithelization and collagenization; however it retards scar formation and granulation (*Diwan et al.*, 1983).

*Tridax procumbens* antagonized anti-epithelization and tensile strength depressing effect of dexamrthasone

without affecting its anticontraction and antigranulation action (*Diwan et al*, 1983). *Ficus glomerata* Roxb leaves, bark and fruit are employed in native medicine. The bark is given as an astringent and as a wash for wounds. It is also employed to remove the poison from wounds made by a tiger or cat. It has been reported to possess antidiarrhoeal (*Manda et al.*, 1997) antibacterial hypoglycemic and antifilarial activities. (*Mishra et al.*, 2005) The leaves and bark extracts of plant has been also reported for the anti-inflammatory activity (*Rachelw et al.*, 2003). *Leucas aspera* Linn. (Family: *Lamiaceae*) commonly known as 'Thumbai' is distributed throughout India from the Himalayas down to Ceylon. The plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperients, diaphoretic, and insecticide. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. In Nigeria extracts or exudated of the plant are used as ear drops and in the treatment of boils, fore and promoting wound healing (*Igoli et al.*, 2005, *Annon*, 2005).

## Scope and plan of work

### Scope

Wound is commonly infected with beta hemolytic *Streptococcus*, methicillin resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*.

Wide ranges of antibiotics are being employed at present for treating wound infection. Although antibiotics are showing good results but microbes are gaining resistant to these antibiotics. Hence plants having medicinal properties are used for the treatment.

For the present study the antimicrobial activity of

- *Tridax procumbens* (whole plant)
- *Ficus glomerata* ( bark)
- *Leucas aspera* (whole plant)

An Indian medicine is analyzed against beta hemolytic *streptococci*.

### Plan of work

- Collection of pus sample
- Isolation of -hemolytic *Streptococci*
- Identification of -hemolytic *Streptococci*
- Selection of medicinal plant
- Processing of medicinal plant
- Water and alcohol extract collection
- Organic fraction collection
- Screening of antibacterial activity
- Phytochemical analysis
- Thin Layer Chromatography (TLC).

## Materials and Methods

### Sample collection

Pus samples were collected from clinically diagnosed cases of wound infected persons.

### Plant chosen

- *Tridax procumbens* Linn
- *Leucas aspera*
- *Ficus glomerata* Roxb

These plants materials chosen for antibacterial activity studies

### Microbe chosen

#### Clinical isolates

Beta hemolytic streptococci isolated from the wound infected patients were used as test organisms.

### Samples

Pus samples were collected from clinically diagnosed wound infected patients; inpatients as well as out patients who were coming to the hospital in Vandhavasi.

### Source of plant materials

Good quality of plants of *Tridax procumbens* and *Leucas aspera*, bark of *Ficus glomerata* was collected locally and identified.

### Clinical sample collection

Pus sample were collected in a swab method (sterile swab) from clinically diagnosed patients. After collection of samples, the swab was transported to the lab by using sterile test tube.

### Culture of the sample to look for bacterial etiology Isolation and identification of -hemolytic *Streptococci*

*Streptococcus pyogenes* is one of the gram positive bacteria occurs in chains of varying length. Blood agar media were used to isolate *Streptococcus pyogenes*.

A loopfull of culture from clinical sample was taken and inoculated on blood agar and was incubated at 37°C for 24 hours under aerobic condition. Beta hemolytic colonies were observed on blood. Beta hemolytic colonies were selected from blood agar medium and were inoculated on other selective media like Islam's medium. Incubated aerobically at 37°C for 24 hours and

were looked for specific colony morphology which would confirm the isolation of *Streptococcus pyogenes*. *Streptococcus pyogenes* growth was observed in selective and differential medium and the results were tabulated.

### Identification of -hemolytic *Streptococci*

Selected colonies from selective and differential media were subjected to macroscopy, microscopy and biochemical tests for identification.

### Microscopic observations

Microscopic observations like size, shape and motility reveal the availability of different morphological characters among microorganisms. Simple staining, gram staining and hanging drop methods were done to look for their shape, grams nature and motility of the isolate respectively (Henry, 1994).

### Grams nature

Gram staining was performed to look for the grams nature of the isolate. A purple coloured cell retains gram's crystal violet and was called gram positive bacterium. Pink coloured cells lost primary stain and picked up safranin colour and were called as gram negative bacterium.

### Motility

Bacteria were motile by their flagella. The number and location of which vary among different species. Motility can be observed directly by hanging drop technique i.e., by placing a drop of culture on a microscopic slide and looked under microscope by keeping them inverted.

### Biochemical tests

Physiological and metabolic characteristics of the microorganisms were assessed through biochemical tests. These characteristics are very useful because they are directly related to the nature and activity of microbial enzymes and transport proteins. Proteins are gene products. Analysis of these characteristics provides an indirect comparison of microbial genomes. The following tests were used to characterize microbial enzymes and proteins.

### Indole test (I)

Indole is one of the degradation products of the metabolism of the amino acid tryptophan. Indole test was performed by inoculating the test organism on peptone water. Bacteria that possess the enzyme

tryptophanase are capable of cleaving tryptophan, producing indole, pyruvic acid and ammonia. The presence of indole is detectable by adding Kovac's reagent. Indole is extracted from a medium into the reagent layer by the acidified butonal component and forms a complex with the *p*-dimethyl amino benzaldehyde, yielding a cherry red colour.

Tryptophan → Indole + Pyruvic acid + Ammonia  
*p*-dimethyl amino benzaldehyde+indole→ quinoidol red violet compound

A loop full of test organism was inoculated on peptone water and incubated for 24 hours at 37°C. Indole can be detected by producing cherry red colored ring after adding Kovac's Indole Reagent (Para-dimethyl amino benzaldehyde) on peptone water inoculated with the microbe. The results were recorded (Koneman *et al.*, 1998).

### Methyl red test (MR)

This method was used to differentiate Enterobacteriaceae members based on mixed acid fermentations. Mixed acid fermentation of the test organism was detected by performing MR test. MR test is a valuable one for identifying bacterial species that produce strong acid from glucose. Methyl red is a pH indicator with a range between 6 (yellow) and 4.4 (red). The pH at which methyl red detects acid is considerably lower than the pH of other indicators used in bacteriological culture media. Thus to produce a colour change, the test organism must produce large quantities of acid from the carbohydrate substrate being used.

Methyl red test is a quantitative test for acid production, requiring the organism to produce strong acids (lactic, formic, acetic and pyruvic acids) from glucose through mixed acid fermentation pathway.

Glucose-----→pyruvic acid -----→ mixed acid+CO<sub>2</sub>  
 Methyl red -----→methyl red  
 (Yellow) (Red)

MR – VP broth was inoculated with a test organism and incubated at 37°C for 24-48 hours. 5 drops of methyl red reagent was added directly to the broth and observed for the development of red colour. Formation of red colour indicates positive result. Methyl red reagent changes its colour only when the pH comes to 4.4 (Koneman *et al.*, 1998). The results were recorded and used for identification of isolates.

### Voges Proskauer test (VP)

The VP test was performed to detect acetyl methyl carbinol (acetoin). It is the product of glucose

fermentation by butylenes glycol pathway. Pyruvate is formed during the fermentative degradation of glucose, which is further metabolized by bacterial enzymes through butylene glycol path way and produce acetoin. In the presence of atmospheric oxygen and 40% KOH, acetoin is converted in to diacetyl and alpha naphthol serve as a catalyst to bring out a red complex.

Glucose -----→ pyruvic acid-----  
 →acetoin + CO<sub>2</sub>↑  
 KOH  
 Acetoin -----→ di acetyl  
 oxygen

Diacetyl +α naphthol + guanidine group-----→ pink to red complex

Sterilized MR – VP broth was inoculated with the test organisms and incubated at 37°C for 24 to 48 hours. Acetoin was detected by adding 0.5 ml of alpha naphthol followed by 0.5 ml of potassium hydroxide solution to the MR-VP medium inoculated & incubated with the test organism.. The tube was shaken gently then allowed to remain undisturbed for 10 to 15 minutes before the reaction is read. Formation of brownish red colour indicated positive result (Koneman *et al.*, 1998). The result was recorded.

### Citrate utilization test (C)

The principle of the citrate utilization test is to determine the ability of an organism to utilize sodium citrate as the sole source of carbon for metabolism and growth. The measurement of this characteristic is important in the identification of many members of the Enterobacteriaceae. The utilization of citrate by a test bacterium is detected in citrate by the production of alkaline by products. The medium includes sodium citrate, an anion, as the sole source of carbon and ammonium phosphate as a sole source of nitrogen. Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonia, leading to alkalization of the medium from conversion of the NH<sub>3</sub><sup>2+</sup> to NH<sub>4</sub>OH.

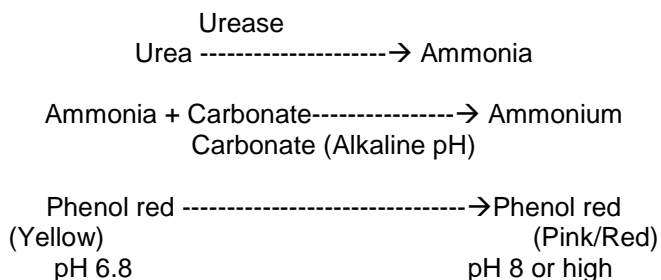
Sodium citrate NH<sub>3</sub><sup>2+</sup> NH<sub>4</sub>OH  
 Bromothymol blue -----→  
 Bromothymol blue (Green)  
 (Blue)  
 pH 7 pH 8 or high

This metabolism leads to the formation of alkaline end product ammonium hydroxide from ammonia. Bromothymol blue detects the pH change of the medium by changing the colour. Test organism was streaked on sterilized simmons citrate agar slant and incubated at 37°C for 24 to 48 hours. The formation of blue color in the test medium indicates positive citrate utilization result

(Koneman *et al.*, 1998). The result was recorded and used for identification of the isolates.

### Urease production test (U)

This test is used to differentiate microorganisms based on urea hydrolysis. Urea is a diamide of carbonic acid. Urease, the enzyme possessed by the bacterium hydrolyzes urea and releases ammonia and carbon dioxide. Ammonia reacts in solution to form ammonium carbonate, which is alkaline leading to the increase in the pH. Phenol red incorporated in the medium changes its colour from yellow to red in alkaline pH.



Christenson urea agar slant was inoculated with the test organisms and incubated at 37°C for 24 to 48 hours. Development of pink color slant indicates positive reaction (Koneman *et al.*, 1998). The result was recorded and used for identification of the isolates.

### Nitrate reduction test (N)

Nitrate reduction is a key factor in identifying microorganisms. The enzyme nitrate reductase possessed by the organisms reduce nitrate to nitrites. In some microorganisms nitrate is further reduced to ammonia and molecular nitrogen and this process is called denitrification. Presence of nitrite is tested by addition of alpha naphthalamine and Sulphanilic acid in acid environment with the formation of with the formation of a red diazonium dye, p sulfo benzene-azo-alpha-naphthylamine. Nitrate broth was inoculated with the test organism and incubated at 37°C for 18-24 hrs. 1ml of reagent A (alpha naphthalamine) and reagent B (Sulphanilic acid) were added to the test medium at the end of incubation. Organisms demonstrating nitrate reduction have the capability to extracting oxygen from nitrates to form nitrites and other reduction products. The chemical equation is

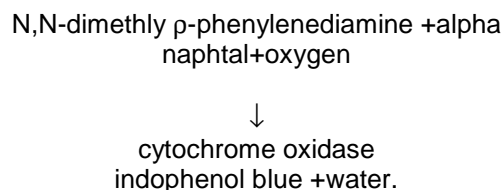


Development of red colour within 30 seconds indicates the presence of nitrites and represents positive reaction for nitrate reduction. If the colour is not observed after the addition of these reagents pinch of zinc powder is to be added. Development of red colour now indicates, the

test is negative. If there is no colour change that indicates further reduction of nitrites (Koneman *et al.*, 1998). The result was observed and used for identification of the isolates.

### Cytochrome oxidase activity

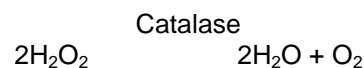
Bacterial species having cytochrome oxidase activity was detected by making use of p-phenylene diamine dihydrochloride that substitutes for oxygen as artificial electron acceptor and develop deep blue colour in the presence of oxygen.



Well grown colony of test organism was smeared on oxidase disc and looked for the development of deep blue colour within 10 seconds. Development of deep blue colour indicates the positive result (Koneman *et al.*, 1998). The result was observed and used for identification of the isolates.

### Catalase test

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.



24-hours old test organism was taken in a sterile wooden stick or a glass rod and was immersed in a hydrogen peroxide solution. Development of immediate bubbling indicates that the organism posses catalase enzyme (Koneman *et al.*, 1998). The result was observed and used for identification of the isolates.

### Antibiotic sensitivity assay

#### Materials

#### Test organism

*Escherichia coli* and *Shigella sp.* was the prevalent organism among the pathogens isolated from diarrhoeal patients. Based on the prevalence - pattern, these three organisms were selected as test organism.

#### Preparation of inoculums

Clinical isolates and referral strains were inoculated in nutrient broth and incubated at 37°C for 4 hours in an

incubatory shaker (Orbitek). This 4 hours culture was used for anti - bacterial activity test.

### Determination of Antibacterial activity

Disc diffusion method was followed (Bauer *et al.*, 1966) to look for anti - bacterial activity. Petri plates containing 20 ml of Mueller Hinton agar were seeded with 4 hours fresh culture of clinical isolate and referral standard individually. By making use of template drawn commercial antibiotic discs were dispensed on the solidified Mueller Hinton agar. This was incubated at 37° C for 24 hours in an incubator (Rands SBC) and was looked for the development of clearance / inhibition zones around the antibiotic disc. The zone of inhibition was measured by making use of antibiotic zone scale (Hi-Media) and the results were recorded.

### Medicinal plants and their antimicrobial activity

#### Plant material – collection and process

Based on ancient and recent literature, traditional use and Siddha and Ayurvedha system of medicinal plants (*Tridax procumbens*, *Leucas aspera*, *Ficus glomerata*) were chosen to look for their anti - bacterial activity.

#### Extraction

Known quantity of these plant extracts coarse powder was taken and the crude extract was made by cold extraction with water and 100 % ethyl alcohol. Cold extraction was repeated 3 or 4 times and the crude aqueous extracts were collected. The extracts were dried in a vacuum desiccator and were stored in a sterile container for further use (Kelmanson *et al.*, 2000). Coding was given to the crude aqueous extracts.

#### Antimicrobial activity test

##### Test microorganisms

hemolytic *Streptococci* isolated from the wound infection were used as test organism. Standard referral *Escherichia coli* strain obtained from Microbial Type Culture Collection Centre, Chandigarh was also used for reference purpose.

#### Preparation of disc

Known quantity of extracts or fractions was dissolved of DMSO: Methanol of 1:1 ratio. This in turn was diluted with equal volume of phosphate buffered saline (PBS pH 7). It was then filter sterilized by making use of soteric syringe filter of pore size 0.22µm. Sterile discs of 6 mm diameter (Hi-Media) were loaded with various concentrations of extracts and fractions and were dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and used as

negative control. Ox tetracycline loaded Hi-Media discs were used as positive control.

### Preparation of inoculums

Clinical isolates and referral strains were inoculated in nutrient broth and incubated at 37°C for 4 hours in a shaker (Orbitech, Scigenics, India) and was used for anti - bacterial activity test and to look for the MIC of various extracts and fractions.

### Determination of Antibacterial activity

Disc diffusion method was followed (Bauer *et al.*, 1966) to determine the anti - bacterial activity of various extracts and fractions. Petriplates containing 20 ml of Mueller Hinton agar were seeded with 4 hours old fresh culture of clinical isolates and referral strains. By making use of template drawn extracts and fractions loaded discs were dispensed on the solidified Mueller Hinton agar with test organisms. Ox tetracycline antibiotic disc obtained from M/s Hi-Media laboratories Ltd, Mumbai was used as a positive control and solvent loaded discs were used as a negative control. This was incubated at 37° C for 24 hours in an incubator (Rands SBC). The test was performed in triplicates. The zone of inhibition was measured by making use of Antibiotic zone scale (Hi - media).

### Determination of Minimum Inhibitory Concentration

Agar dilution method was used to find out Minimal Inhibitory Concentration (NCCLS, 1993). Stock concentration of various plant extract was prepared by making use of DMSO : Methanol, in the ratio of 1:1 which in turn was diluted with equal volume of phosphate buffered saline, pH 7. Mueller Hinton agar was prepared, sterilized and kept ready in molten condition. 20ml of the molten media was taken and was mixed with known concentration of different extracts / fractions and were added in different tubes. This mixture was swirled carefully for complete mixing of extract and media and poured on to the plate. After getting solidified it was inoculated with the test organism and standard organism. The plates were incubated at 37°C for 24 hours. MIC was recorded based on the growth of the organisms.

### Phytochemistry

Phytoconstituents are responsible for these activities. Knowledge of pharmacologically active compound is necessary to develop good and effective medicine from plants. Medicinal plants are the good source of macromolecules and secondary metabolites.

**Phytochemical screening**

Chemical tests were carried out using the aqueous extracts from plants and or the powdered specimens, using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

**Test for alkaloids**

**Mayer's test (Evans, 1997):** To a few ml of the filtrates, a drop of Mayer's reagent was added by the side of the test tube. A creamy or white precipitate indicates the test is positive.

**Test for carbohydrates**

**Benedict's test:** To 0.5 ml of the filtrate, 0.5 ml of Benedict's reagent was added. The mixture was heated on boiling water bath for 2 min. A characteristic red coloured precipitate indicates the presence of sugar.

**Test for saponins**

The extract was diluted with distilled water and made up to 20 ml. The suspension was shaken in a graduated cylinder for 15 min. 2 cm layer of foam indicates the presence of saponins.

**Test for tannins:**

About 0.5 mg of dried powdered samples was boiled in 20 ml of water in test tubes then filtered. A few drops of 0.1 % ferric chloride was added and observed for brownish green or blue black colouration.

**Test for flavonoids:**

To 5 ml of the dilute ammonia solution a portion of the aqueous extract was added, followed by addition of concentrated sulphuric acid. Appearance of yellow colouration indicates the presence of flavonoids.

**Test for terpenoids (Salkowski test):**

5 ml of the extract was mixed with 2 ml of chloroform and concentrated sulphuric acid to form a layer. A reddish brown coloration of the interface showed the presence of terpenoids.

**Qualitative analysis of alkaloids.**

1ml of the extract was taken and 1ml of Mayer's reagent was added to that in a test tube and this mixture was allowed to stand for some time to develop colour and results were recorded. Development of cream colour indicates the presence of alkaloids.

**Qualitative analysis of steroids**

To 1ml of the extract, 1ml of sulphuric acid was added in a tube and it was allowed to stand for some time. The colour developed was recorded. Development of reddish brown colour indicates the presence of steroids.

**Qualitative analysis of glycosides**

To 1ml of the extract, 1ml of the – naphthol was added to which chloroform was added along the sides and it was looked for the development of colour and the result was recorded. Development of violet colour indicates the presence of glycosides.

**Qualitative analysis of secondary metabolites by TLC (Anonymous, 1998)**

TLC was performed by making use of readymade silica coated aluminium plate supplied by M/s Qualigens chemicals Pvt Ltd, Mumbai. The thickness of the TLC plate is about 0.25mm. TLC plate was activated by heating at 110°C for 10 minutes in a hot air oven and allowed to cool.

**Qualitative analysis of triterpenoids**

To 1ml of the warm extract 1ml each of tin and thionyl chloride were added. It was allowed to develop colour and the colour was noted. Development of pink colour shows the presence of triterpenoids.

**Qualitative analysis of saponins**

1 ml of the extract was taken with 1 ml of water, shaken and was allowed to stand for some time to develop froth. Formation of stable froth indicates the presence of saponin.

**Application of sample**

20x20cm size TLC plate was taken. Starting line was drawn 15 mm above the lower edge using marking pencil. Plant extract was applied on the starting line as spot by making use of capillary tube. All the extracts and fractions of the single plant were applied in a single plate with 15 mm space in between. Spot was made up to 4mm diameter and was allowed to cool at room temperature.

**Development of chromatogram**

TLC chamber was saturated with solvent mixture which contains chloroform: ethyl acetate: formic acid in the ratio of 5:4:1. The TLC plate was placed in the saturated chamber and the chromatogram was allowed to run. The chamber was closed and the chromatogram was

developed at room temperature by allowing the solvent to ascend the specified distance. TLC plate was removed from the chamber and position of the solvent front was marked. Solvent available in the plate was allowed to evaporate at room temperature.

### Observation

TLC plate was observed in daylight initially. Sulphuric acid reagent was sprayed on the plate. Plate was placed in a hot air oven and heated at 60°C for 10 minutes, the coloured spot developed was observed. The distance of each spot to the point of application was recorded. Rf value was calculated by making use of the formula.

$$R_f = \frac{\text{Distance of the spot}}{\text{Distance of the solvent travelled}}$$

### Results and Discussion

Skin is the most understandable region in the body. It protects human from external transient microbial flora. Symptoms due to bacterial skin infections are inflammation and opsonization. This results in the formation of wound. All inflammatory wounds are created by microorganisms such as Beta hemolytic *Streptococcus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella spp.*,

Wound healing property of an individual depends upon nutritional status, hygienic condition and virulence property of the pathogen. A total of 25 samples were collected from patients of Government hospital, Vandhavasi for a period of two months, samples were categorized based on sex. Results revealed that more number of samples were collected from males (n=14)

All the samples were subjected for microbiological examination preliminary by microscopy and also by macroscopic procedures. Selective and differential medium also used for the cultivation of bacteria. Totally 7 Beta hemolytic *Streptococci* were isolated from 25 samples. This indicates that Beta hemolytic *Streptococcus* causes frequent wound infection compared to other bacteria

Bacterial isolates of wound infection were identified by colony morphology on Blood agar, Nutrient agar etc., and also by using biochemical tests like, Indole, Methyl red, Voges proskauer etc

Beta hemolytic *Streptococcus* was identified from clinical sample. When we note the prevalence bacterial etiology *Streptococcus pyogenes* (28%) shows higher incidence followed by *Staphylococcus aureus*,

*Pseudomonas aeruginosa*. Maximum numbers of gram positive organisms were resistant to amoxicillin, gentamycin, tetracycline and chloramphenicol.

Brindha.P *et al.*, (2008) proved that wound healing potential of a herbal formulation consisting of *Tridax procumbens* and *Ficus racemosa* Chika.C *et al.*, (2007) showed antibacterial activities of *Leucas aspera*. Antibiotic sensitivity assay was performed by making use of Kirby and Bauer disc diffusion method.

*Tridax procumbens*, *Leucas aspera* and *Ficus racemosa* are used as the remedy for boils, wounds of human. Various extracts and fractions were collected from plants of *Tridax procumbens*, *Leucas aspera* and bark from *Ficus racemosa* by making use of solvents like water, alcohol, hexane, chloroform. Results show that all the extract and fractions produced zone of inhibition.

Water extract of *Leucas aspera* showed higher activity against *Streptococcus pyogenes* compared to other extracts. Zone of inhibition against *Streptococcus pyogenes* ranges between 12mm to 18mm. Perumal samy *et al.*, (1999) showed similar kind of activity against *Streptococcus pyogenes* by using *Leucas aspera*. *Leucas aspera* showed significant zone of inhibition when tested against *Streptococcus* and *Staphylococcus*. The large zone of inhibition exhibited by the extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa* justified their use by traditional medical practitioners in the treatment of sores, boils and open wounds. *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been implicated in cases of boils, sores and wounds (Braude, 1982), the low MIC exhibited by the extract on *Staphylococcus aureus* is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infection due to this microorganism, especially as they frequently develop resistance to known antibiotics.

Water extract of *Tridax procumbens* showed activity against *Streptococcus pyogenes*. This plant produced zone of inhibition against *Streptococcus pyogenes* ranges between 12mm to 16mm. Traditionally village peoples use this plant along with turmeric powder and salt for the treatment of boil and soft tissue infections. Our results also supported by Kumar *et al* (2007). They reported that extracts of *Tridax procumbens* inhibit the growth of boil causing organisms like *Propionibacterium acnes* and *Staphylococcus aureus*

Shivakumar .H. *et al.*, (2006) showed that anti-inflammatory activity of *Ficus glomerata* Linn fruit. In the present study water extract and chloroform extract of *Ficus glomerata* bark have zone of inhibition against



*Streptococcus pyogenes* ranges between 10 mm to 14mm

This phytochemical screening of the plants was screened by making use of standard methods. It shows tannin; terpenoids were present in all extract of *Ficus glomerata*. Flavonoids are present in hexane and chloroform extract. Flavonoids have been shown to affect various biological functions like capillary permeability inflammatory response inhibition of enzymes, receptors and carries etc Afanasev *et al.*, (1989). Alkaloids and quinone were present in all extract and fractions of *Leucas aspera* and *Tridax procumbens*. Alkaloid and quinone may be responsible for maximum zone of inhibition.

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