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Original Research Article

Phytochemical and antibacterial activity of *Vitex negundo, Leucas aspera* and *Abutilon indicum* against wound pathogens

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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 threating complication wounds are commonly infects *Streptococcus pyogenes*, S*taphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*. Among these *Streptococcus pyogenes* shows highest predominance. This work is mainly focused to know the antimicrobial activity of *Vitex negundo, Leucas aspera, and Abutilon indicum* against wound pathogens. Totally 25 wound samples were collected from Government hospital, Cheyyar. The infectious organisms *Streptococcus pyogenes*, S*taphylococcus aureus, Pseudomonas aeruginosa*, *E.coli*., 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 *Vitex negundo, Leucas aspera, Abutilon indicum*. Water, acetone, 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,* S*taphylococcus aureus*, *Pseudomonas aeruginosa*, *E.coli*.

Keywords: Wounds, pathogens, Herbal medicines.

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 neropatic 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 Beta hemolytic *Streptococci*, *Staphylococcus aureus, Pseudomonas aeruginosa* and *E.coli* have been most frequently cited as the cause of delayed wound healing and infection.
Roots and leaves used in eczema, ringworm and other skin diseases, liver disorders, spleen enlargement, rheumatic pain, gout, abscess, backache; seeds used as vermicide. It is an effective on colds, asthuma, cancer and asthuma attack.

Vitex negundo 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, 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).

Leucas aspera is reported to have antifungal, prostaglandin inhibitory, antioxidant, antimicrobial, antinociceptive and cytotoxic activities. *Leucas aspera* is used in the traditional medicine of the Philippines to treat scorpion bites. It is also an antipyretic; it is a herb that has the ability to help reduce fevers.

In some forms of traditional medicine, the steam formed by crushing the Samoolam, also known as the plant's flowers, seeds, roots, berries, bark or leaves, can be inhaled to help treat nasal congestion, coughing, cold, headache and fever.

In traditional medicine, *A. indicum* various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, and analgesic and to treat leprosy, ulcers, headaches, gonorrhea, and bladder infection.The whole plant is uprooted, dried and is powdered. In ancient days, maidens were made to consume a spoonful of this powder with a spoonful of honey, once in a day, for 6 months until the day of marriage, for safe and quick pregnancy.

The aerial parts of *A.indicum* extract also showed activity against *Mycobacterium smegmatis* and *Staphylococcus aureus*, while the aqueous extract showed no antimicrobial activity. None of the tested extracts was active against the yeasts, *Candida albicans*, *Candida tropicalis* and *Rhodotorula rubra* or the fungi: *Aspergillus flavus, Aspergillus niger, Mucor sp*. and *Trichophyton rubrum.* (Taddei A, and Rosas-Romero AJ 2000).

Antibacterial activities of aqueous residues of 16 different ethnomedicinal plants have been studied. The effect of the aqueous extract at two different weights of plant residues, 30 and 40 mg, were tested against three gram positive bacteria and seven Gram negative bacteria by the filter paper disc diffusion method. Among the tested plants, Cleome gynandropsis and Ageratum conyzoides showed a significant control of the growth of *Alkaligens viscolactis, Klebsiella aerogenas, Bacillus cereus* and *Streptococcus pyogens*. The maximum inhibitions were observed in *Tridax procumbens, Cleome viscosa, Acalypha indica* . (Perumal Samy *et al.*, 1999)

The present study was carried out to assess the effect of chloroform insoluble fraction of ethanolic extract of *A.indicum* against D-Galactosamine/ Lipopolysaccharide (D-GalN/LPS)-induced hepatitis. There was a decline in the activities of enzymic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione s-transferase and the levels of non-enzymic antioxidants namely reduced glutathione, vitamin C and vitamin E. These biochemical alterations were normalized upon pretreatment with TP extract (Kumar *et al.*, 2007).

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)

Materials and Methods

Sample collection

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

Plant chosen

- *Vitex negundo*

- *Leucas aspera*
- *Abutilon indicum*

These plants materials chosen for antibacterial activity studies.

Microbes Chosen

Clinical isolates

Beta hemolytic *streptococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E.co*li 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 outpatients who were coming to the hospital in Cheyyar.

Source of plant materials

Good quality of plants of *Vitex nedungo*, *Leucas aspera*, and *Abutilon indicum* was collected locally and identified.

Isolation of bacteria from samples

The pus sample were processed in such a way that they were streaked on the prepared nutrient agar,Blood agar, Mannitol salt agar, Cetrimide agar and EMB agar plates aseptically.Then by using inoculation needle, it was streaked for the growth of isolated colonies and then the plates were incubated at 37° C for 24 hrs for bacteria. After 24 hrs the colonies grown on the plates were examined for their morphology, haemolysis and the same type of colonies was used for Gram's staining.

Beta-hemolytic activity in Blood agar indicate the presence of *Streptococcus* sp. The golden yellow colonies in Mannitol salt agar medium indicate the presence of *S.aureus*.The green colour colonies in cetrimide agar medium indicate the presence of *Pseudomonas* sp. The metalic sheen appearence in EMB medium indicate the presence of *E.coli*.

The morphology of each type of colony was examined and the results were noted. Each type of colony was aseptically taken from the plate and a thin smear was prepared on a glass slide. The preparation was heat fixed, Gram stained and observed under microscope. The results were noted.

The same type of colony was simultaneously taken from the plate aseptically and streaked on the prepared nutrient agar plate. Then the plates were incubated at 37°C for 24 hrs. After one day, the results were noted for their colony morphology and pigment production and also the colonies grown on the plate were used for performing biochemical tests and antibiotic sensitivity tests.

Preparation of plant extracts

Aqueous extracts

Fifty grams of each of the dried powder plant materials (leaf and stem) were suspended in 200 ml of sterile double distilled water (1:4).They was kept for three days at room temperature (31º c) for complete extraction. After three days, the extracts were filtered through Whatman No. 1 filter paper. This crude extract was poured in labeled sterile universal bottles and kept in refrigerator at 4 º c. (Kelmanson *et al.*, 2000).

Solvent extracts

Fifty gram of each of the dried powdered materials (leaves, stems) were soaked separately in 200 ml of each of the solvents viz. Acetone, Chloroform, in soxhlet apparatus for 72 hr at 31º c until complete exhaustion of the material. Each mixture was stirred at every 24 hrs using a sterile glass rod. At the end of 72 hr, each extract was passed through Whatman No 1 filter paper and the filtrates were concentrated in vaccum rotary evaporator at 60 º C in order to reduce the volume. The paste like extracts were stored in labeled screw capped bottles and kept in refrigerator at 4 º C. Each of the extract was individually diluted using minimal amounts of the extracting solvent prior to use.

Antibiotic sensitivity assay

Test organism

Beta-hemolytic *Streptococci*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* was the prevalent organism among the pathogens isolated from wound infected patients. Based on the prevalence - pattern, these four organisms were selected as test organism.

Preparation of inoculums

Clinical isolates and referral strains were inoculated in nutrient broth and incubated at 37^{O}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 Test ((Bauer *et al.*, 1966)

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 sortorious syringe filter of pore size 0.22µm.

Circular discs mm diameter were prepared from Whatman No 1 filter paper and sterilized in an autoclave. These paper discs were impregnated with test compounds (plant extract) in the respective solvents for overnight and placed on nutrient agar plates seeded with the test bacterium. The plates were incubated at 37 º c for 24 hrs, After 24 hrs the zone of inhibition around each disc was measured and the diameter was recorded. Gentamycin (10 mcg/disc) was used as the reference. A negative control was prepared using only the solvent used for extraction and kept for comparison. The tests were repeated 4 times to ensure reliability of the result.

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 α – napthol 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 (Annonymous, 1998)

TLC was performed by making use of readymade silica coated aluminium plate. The thickness of the TLC plate is about 0.25mm. TLC plate was activated by heating at 110ºC 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, shaked 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 assent 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 the formula.

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

Results and Discussion

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

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 patience of Government hospital, Cheyyar for a period of the two months, samples were categorized based on sex. Result revealed that more number of samples were collected from male (n=14) (Table 1).

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*, 5 *Staphylococcus aureus,* 4 *Pseudomonas aeruginosa and* 4 *E.coli* were isolated from 25 samples. This indicates these bacteria causes frequent wound infection compare to other bacteria.

Bacterial isolates of wound infection were identified by colony morphology on Blood agar, Nutrient agar,Mannitol salt agar, Cetrimide agar, EMB etc., and also by using biochemical tests like, Indole, Methyl red,. Voges proskauer etc., (Table 2a,b,c,d).

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 (20%), Pseudomonas aeruginosa (16%), E.coli(16*%).(Table 3). Maximum numbers of Gram positive organisms were resistance to amoxycillin, gentamycin, tetracycline and chloramphenicol.

Brindha.P *et al*., (2008) proved that wound healing potential of a herbal formulation consisting of *Vitex negundo and Abutilon indicum.*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.

Vitex negundo,Leucas aspera and *Abutilon indicum* are used as the remedy for boils, wounds of human.Various extracts and fractions were collected from plants of *Vitex negundo, Leucas aspera* and *Abutilon indicum* by making use of solvents like water,acetone, chloroform (Table- 4, 5 & 6). Result shows that all the extract and fractions produced zone of inhibition.

Acetone of *Vitex negundo* showed activity against *Streptococcus pyogenes, S.aureus, Ps.aeruginosa and E.coli compared to other extracts.* This plant produced zone of inhibition against *Streptococcus pyogenes* ranges between 12mm to 18mm, *Staphylococcus aureus* (13mm to 19mm*), Pseudomonas aeruginosa* (14mm to 19mm) and *E.coli* (13mm to 19mm).Traditionally village peoples uses this plant along with turmeric powder and salt for the treatment of boil and soft tissue infections.Our result also supported by Kumar *et al* (2007).they reported that extracts of *Vitex negundo* inhibits the growth of boils casing organisms like *Propionibacterium acnes* and *Staphylococcus aureus* (Table 7 a,b,c&d).

Acetone of *Leucas aspera* showed higher activity against *Streptococcus pyogenes, S.aureus, Ps.aeruginosa and E.coli* compared to other extracts. Zone of inhibition against *Streptococcus pyogenes* ranges between (11mm to 16mm), *Staphylococcus aureus* (12mm to 17mm), *Pseudomonas aeruginosa* (12mm to 17mm) and *E.coli* (13mm to 17mm) . 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,bores 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.(Table 8 a,b,c&d).

Shivakumar .H. et al.,(2006) showed that anti-inflammatory activity of *Abutilon indicum*. In the present study water Acetone of *A.indicum* have zone of inhibition against *Streptococcus pyogenes* ranges between (13mm to 17mm), *Staphylococcus aureus* (12mm to 16mm), Pseudomonas aeruginosa (12mm to 15mm) and *E.coli* (13mm to 16mm). (Table 9 a,b,c&d)

This phytochemical screening of the plants was screened by making use of standard methods.It shows tannin; terpenoids were present in all extract of *Abutilon indicum*. Flavonoids are present in 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 *Vitex negundo*. Alkaloid and quinine may be responsible for maximum zone of inhibition. (Table10, 11&12).

Table:1 Samples collection details and categoriesbased on sex

Sample: pus

S. No	Total No. of samples	Female	Male
1	25	11	14

Table: 2aIdentification features of wound Pathogens based on microscopic and Biochemical tests

CHARACTERIZATION OF beta-hemolytic Streptococci

Microscopy : Gram +ve cocci
Capsule Staining : Positive
Endospore staining : Negative
Colony morphology : Glossy and mucoid colonies
On MacConkey agar : Lactose ferment
On Blood agar : Beta hemolytic with clear zone
Triple sugar iron test : Alkaline sland& Acid but No gas

S. No	Test	SH1	SH2	SH3	SH4	SH5	SH6	SH7
1	Gram staining	+	+	+	+	+	+	+
2	Shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
3	Motility	NM	NM	NM	NM	NM	NM	NM
4	Indole test	-	-	-	-	-	-	-
5	Methyl red test	-	-	-	-	-	-	-
6	Vogesproskaur test	-	-	-	-	-	-	-
7	Citrate utilization test	+	+	+	+	+	+	+
8	Urease test	-	-	-	-	-	-	-
9	Nitrate reduction test	-	-	-	-	-	-	-
10	Catalase tests	-	-	-	-	-	-	-
11	Oxidase test	-	-	-	-	-	-	-
12	Carbohydrate test	-	-	-	-	-	-	-
13	Glucose	+	+	+	+	+	+	+
14	Maltose	+	+	+	+	+	+	+
15	Sucrose	+	+	+	+	+	+	+

M- Motile NM- Non Motile + Positive - Negative

Table: 2b Identification features of wound Pathogens based on microscopic and Biochemical tests

CHARACTERIZATION OF Staphylococcus aureus

Microscopy : Gram +ve cocci
Capsule Staining : Positive
Endospore staining : Negative
Colony morphology : Golden yellow pigment colonies
On MacConkey agar : Lactose ferment smaller colonies
On Blood agar : Beta haemolytic colonies
Triple sugar iron test : Acid slant&Acid butt with no gas

S. No	Test	Sa1	Sa2	Sa3	Sa4	Sa5
1	Gram staining	+	+	+	+	+
2	Shape	Cocci	Cocci	Cocci	Cocci	Cocci
3	Motility	NM	NM	NM	NM	NM
4	Indole test	-	-	-	-	-
5	Methyl red test	+	+	+	+	+
6	Vogesproskaur test	+	+	+	+	+
7	Citrate utilization test	-	-	-	-	-
8	Urease test	-	-	-	-	-
9	Nitrate reduction test	+	+	+	+	+
10	Catalase tests	+	+	+	+	+
11	Oxidase test	-	-	-	-	-
12	Carbohydrate test	+	+	+	+	+
13	Glucose	+	+	+	+	+
14	Maltose	+	+	+	+	+
15	Sucrose	+	+	+	+	+

M- Motile NM- Non Motile + Positive - Negative

Table:2c Identification features of wound Pathogens based on microscopic and Biochemical tests

CHARACTERIZATION OF Pseudomonas aeruginosa

Microscopy : G-ve Rod
Capsule Staining : Negative
Endospore staining : Negative
Colony morphology : Bluish green large opaque irregular colonies
On MacConkey agar : Non lactose fermentor
On Blood agar : Non haemolytic colonies (or) Alpha Haemolytic colonies
Triple sugar iron test : Acid butt – Alkaline slant, Gas +ve, H2S +ve

S. No	Test	Pa1	Pa2	Pa3	Pa4
1	Gram staining	-	-	-	-
2	Shape	Rod	Rod	Rod	Rod
3	Motility	M	M	M	M
4	Indole test	-	-	-	-
5	Methyl red test	-	-	-	-
6	Vogesproskaur test	+	+	+	-
7	Citrate utilization test	-	-	-	-
8	Urease test	-	-	-	-
9	Nitrate reduction test	+	+	+	+
10	Catalase tests	+	+	+	+
11	Oxidase test	+	+	+	+
12	Carbohydrate test	-	-	-	-
13	Glucose	-	-	-	-
14	Maltose	-	-	-	-
15	Sucrose	-	-	-	-

M- Motile NM- Non Motile + Positive - Negative

Table:2d Identification features of wound Pathogens based on microscopic and Biochemical tests

CHARACTERIZATION OF Escherichia coli

Microscopy : G-ve Rods
Capsule Staining : Positive
Endospore Staining : Negative
Colony Morphology : Small colonies in Nutrient agar
On MacConkey agar : Pink colour non mucoid colonies
On Blood agar : Non haemolytic colonies
Triple sugar iron test : Acid butt –Acidslant,Gas production

S. No	Test	Ec1	Ec2	Ec3	Ec4
1	Gram staining	-	-	-	-
2	Shape	Rod	R	R	R
3	Motility	M	M	M	M
4	Indole test	+	+	+	+
5	Methyl red test	+	+	+	+
6	Vogesproskaur test	-	-	-	-
7	Citrate utilization test	-	-	-	-
8	Urease test	+	+	+	+
9	Nitrate reduction test	+	+	+	+
10	Catalase tests	+	+	+	+
11	Oxidase test	-	-	-	-
12	Carbohydrate test	+	+	+	+
13	Glucose	+	+	+	+
14	Maltose	+	+	+	+
15	Sucrose	+	+	+	+

M- Motile NM- Non Motile + Positive - Negative

Table:3 Incidence of bacterial etiology

S. No	Total Sample	Isolated organisms	Number of isolates	% of incidence
1	25	*Streptococcus pyogens*	7	28
		Staphylococcus aureus	5	
		Pseudomonas aeruginosa	4	
		Escherichia coli	4	16

Figure-1

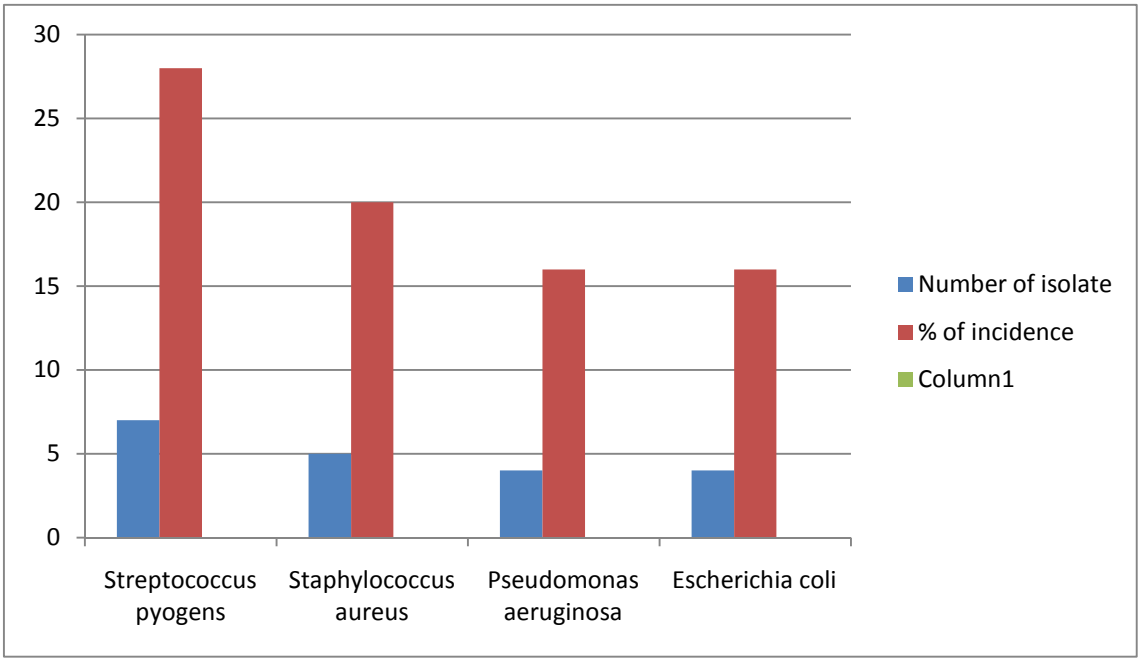


Table:4 Nature of solvent and plant powder (*Vitex negunda*) on Extract preparation

S.No	Extract/fraction	Volume of solvent added (ml)	Powder taken (g) for extraction	After 3 days (cold extraction)	Volume of solvent collection (ml)	Non extractable solid (g)	Solvent Lossed (ml)	Final dry extract weight (g)
1	Water extract	200	50		175	17.3	83.3	11.2
2	Acetone extract	200	50		150	39.5	59.5	8.2
3	chloroform fraction	200	50		120	44	53	4.8

Table: 5 Nature of solvent and plant powder (*Leucas aspara*) on Extract preparation

S.No	Extract/fraction	Volume of solvent added (ml)	Powder taken (g) for extracti	After 3 days (cold extraction)	Volume of solvent collection (ml)	Non extractable solid (g)	Solvent Lossed (ml)	Final dry extract weight (g)
1	Water extract	200	50		140	23.5	88.2	12
2	Acetone extract	200	50		155	37.5	60.8	7.3
3	Chloroform fraction	200	50		145	46	52	5.3

Table: 6Nature of solvent and plant powder (Abutilon indicum) on Extract preparation

S.No	Extract/fraction	Volume of solvent added (ml)	Powder taken (g) for extract	After 3 days (cold extraction)	Volume of solvent collection (ml)	Non extractable solid (g)	Solvent Lossed (ml)	Final dry extract weight (g)
1	Water extract	200	50		150	22.4	177.1	10.2
2	Acetone extract	200	50		165	43.1	62.5	6.3
3	Chloroform fraction	200	50		130	45	53	4.5

Table:7a Antibacterial activity of *Vitex negundo* extracts/fractions against *Streptococcus pyogens*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	12	Nil	10	12	13	14
2	Acetone extract	13	Nil	12	14	16	18
3	Chloroform fraction	12	Nil	13	15	15	16

Negative control - DMSO Positive control - Gentamycin

Table:7b Antibacterial activity of *Vitex negundo* extracts/fractions against *Staphylococcus aureus*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	12	Nil	10	13	14	15
2	Acetone extract	13	Nil	13	15	17	19
3	Chloroform fraction	12	Nil	11	12	12	14

Negative control - DMSO Positive control - Gentamycin

Table:7c Antibacterial activity of *Vitex negundo* extracts/fractions against *Pseudomonas aeruginosa*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	12	Nil	11	13	14	15
2	Acetone extract	13	Nil	14	15	17	19
3	Chloroform fraction	12	Nil	13	14	15	15

Negative control - DMSO Positive control - Gentamycin

Table:7d Antibacterial activity of *Vitex negundo* extracts/fractions against *E.coli*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	12	Nil	11	12	13	14
2	Acetone extract	13	Nil	13	15	17	19
3	Chloroform fraction	12	Nil	10	11	11	13

Negative control - DMSO Positive control - Gentamycin

Figure-2 Antibacterial activity of Vitex negundo

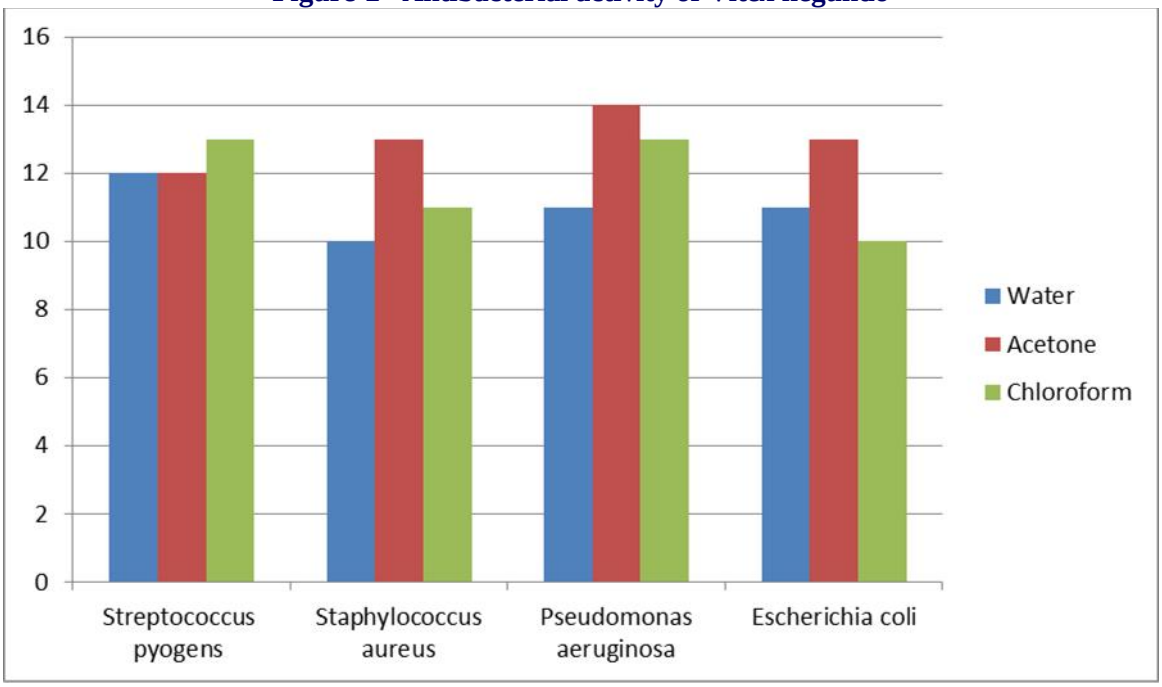


Table:8a

Antibacterial activity of *Leucas aspera* extracts/fractions against *Streptococcus pyogens*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	10	11	11	14
2	Acetone extract	12	Nil	11	12	14	16
3	Chloroform fraction	12	Nil	9	10	12	13

Negative control - DMSO Positive control - Gentamycin

Table:8b Antibacterial activity of *Leucas aspera* extracts/fractions against *Staphylococcus aureus*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	10	12	12	15
2	Acetone extract	12	Nil	12	14	15	17
3	Chloroform fraction	12	Nil	9	11	13	14

Negative control - DMSO Positive control - Gentamycin

Table:8c Antibacterial activity of *Leucas aspera* extracts/fractionsagainst *Pseudomonas aeruginosa*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	11	12	13	15
2	Acetone extract	12	Nil	12	13	15	17
3	Chloroform fraction	12	Nil	9	11	13	14

Negative control - DMSO Positive control - Gentamycin

Table:8d Antibacterial activity of *Leucas aspera* extracts/fractions against *Escherichia coli*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	11	11	13	15
2	Acetone extract	12	Nil	13	13	15	17
3	Chloroform fraction	12	Nil	11	11	13	14

Negative control - DMSO Positive control - Gentamycin

Figure-3 Antibacterial activity of Leucas aspera

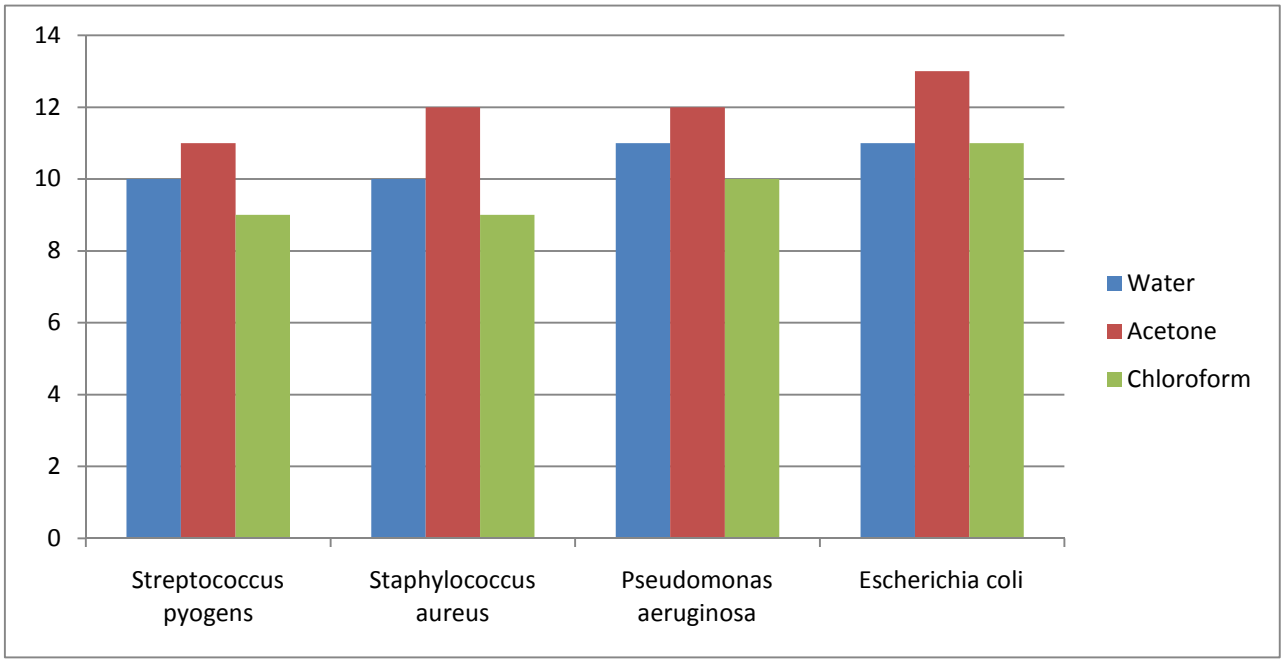


Table:10a ntibacterial activity of *Abutilon indicum* extracts/fractions against *Streptococcus pyogens*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	11	11	12	13
2	Acetone extract	13	Nil	13	14	16	17
3	Chloroform fraction	12	Nil	10	11	11	12

Negative control - DMSO Positive control -Gentamycin

Table:10bAntibacterial activity of *Abutilon indicum* extracts/fractions against *Staphylococcus aureus*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	9	10	11	12
2	Acetone extract	13	Nil	12	14	15	16
3	Chloroform fraction	12	Nil	11	12	12	13

Negative control - DMSO Positive control - Gentamycin

Table:10c Antibacterial activity of *Abutilon indicum* extracts/fractions against *Pseudomonas aeruginosa*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	10	11	12	13
2	Acetone extract	13	Nil	12	13	13	15
3	Chloroform fraction	12	Nil	9	12	12	13

Negative control - DMSO Positive control - Gentamycin

Table:10d Antibacterial activity of *Abutilon indicum* extracts/fractions against *Escherichia coli*

S.No	Extracts	Concentration of extracts/zone of inhibition in mm					
		Positive control	Negative control	200µg/disc	400µg/disc	600µg/disc	800µg/disc
1	Water extract	14	Nil	11	12	13	15
2	Acetone extract	13	Nil	13	14	15	16
3	Chloroform fraction	12	Nil	10	12	12	13

Negative control - DMSO Positive control - Gentamycin

Figure-3Antibacterial activity of Abutilon indicum

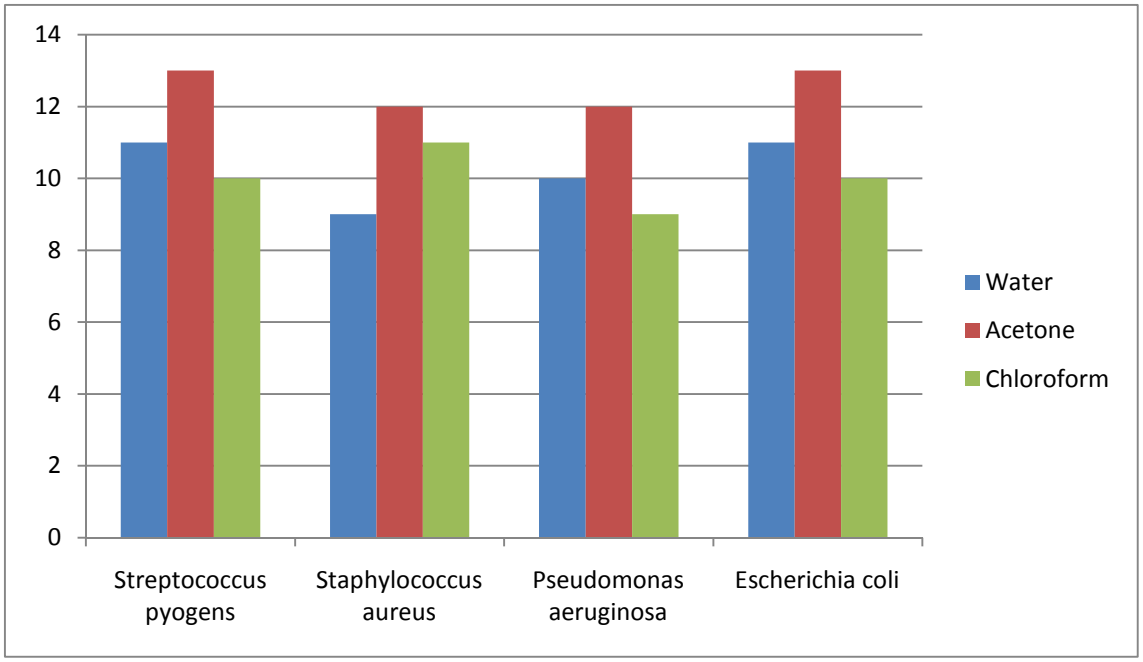


Table:11 Phytochemical screening of *Vitex negundo* extracts and fraction

S No	Tests	Water extract	Acetone extract	Chloroform fraction
1	Terpenoids	+	-	-
2	Flavones	-	-	-
3	Steroids	-	+	-
4	Sugar	-	-	-
5	Alkaloids	+	+	+
6	Quinines	+	+	+
7	Coumarin	-	-	-
8	Tannins	-	-	-
9	Saponins	-	+	+

+ Positive - Negative

Table:12 Phytochemical screening of *Leucas aspera* extracts and fraction

S No	Tests	Water extract	Acetone extract	Chloroform fraction
1	Terpenoids	-	-	-
2	Flavones	+	+	+
3	Steroids	-	+	-
4	Sugar	-	-	-
5	Alkaloids	-	-	-
6	Quinines	+	+	+
7	Coumarin	-	-	-
8	Tannins	-	-	-
9	Saponins	-	+	+

+ Positive
- Negative

Table: 13 Phytochemical screening of Abutilon *indicum* extracts and fraction

S No	Tests	Water extract	Acetone extract	Chloroform fraction
1	Terpenoids	+	+	+
2	Flavones	-	-	+
3	Steroids	-	-	-
4	Sugar	-	-	-
5	Alkaloids	-	-	-
6	Quinines	-	-	-
7	Coumarin	-	-	-
8	Tannins	+	+	+
9	Saponins	+	-	+

+ Positive - Negative

Table:14 Rf value of compounds isolated in TLC (*Vitex negundo*)

S.No	Extracts	Rf value		
		Spot 1	Spot 2	Spot 3
1	Water extract	0.091	0.125	0.353
	Acetone extract	0.092	0.354	0.600
3	Chloroform fraction	0.089	0.500	0.647

Table:15 Rf value of compounds isolated in TLC (*Leucas aspera*)

S.No	Extracts	Rf value		
		Spot 1	Spot 2	Spot 3
1	Water extract	0.098	0.111	0.116
2	Alcohol extract	0.154	0.422	0.677
3	Hexane fraction	0.258	0.420	0.692
4	Chloroform fraction	0.125	0.423	0.612

Table:16 Rf value of compounds isolated in TLC(Abutilon indicum)

S.No	Extracts	Rf value		
		Spot 1	Spot 2	Spot 3
1	Water extract	0.098	0.186	0.343
2	Alcohol extract	0.125	0.354	0.666
3	Hexane fraction	0.091	0.123	0.322
4	Chloroform fraction	0.160	0.256	0.400

Plate -1 Plants

Vitex negunta

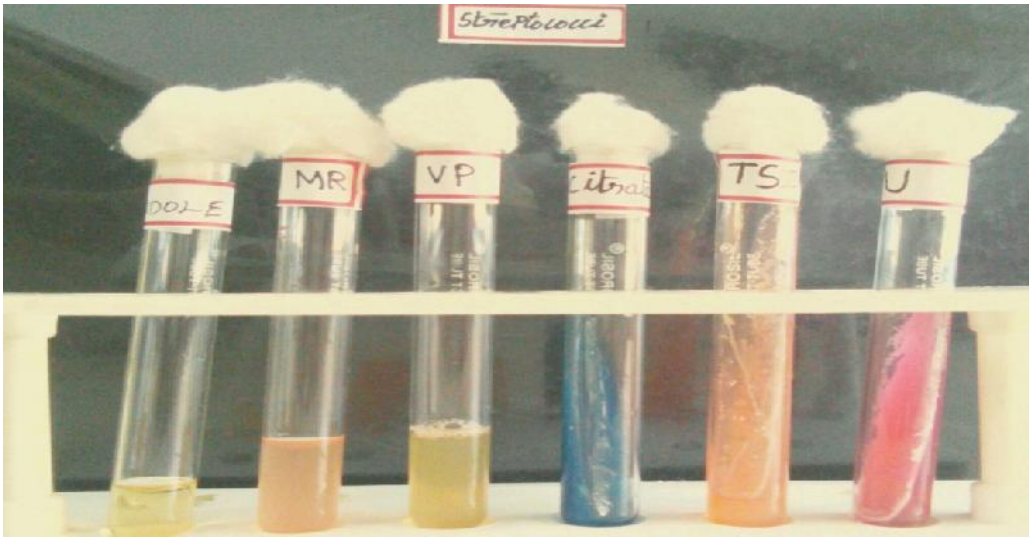
Leucas aspera

Abuliton indicum

Plate-2 isolation and identification of *Streptococcus pyogens*

growth on nutrient agar

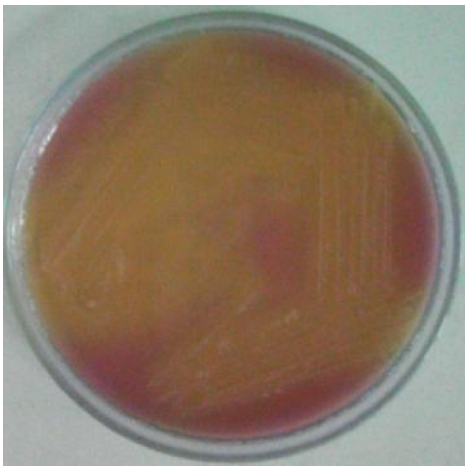
growth on blood agar



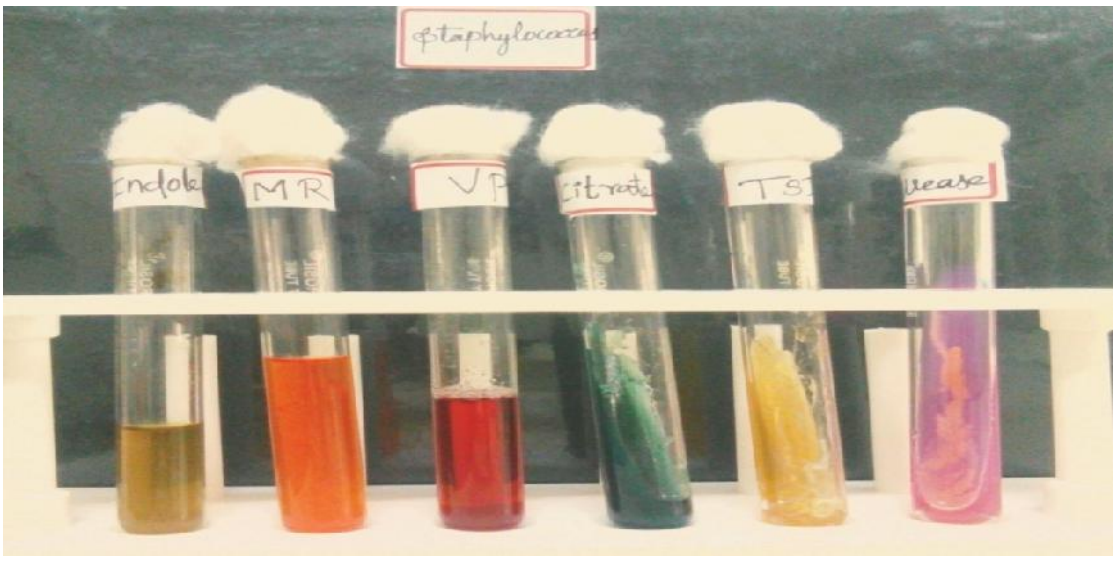
Biochemical test

Plate-3 isolation and identification of *Staphylococcus aureus*

growth on nutrient agar

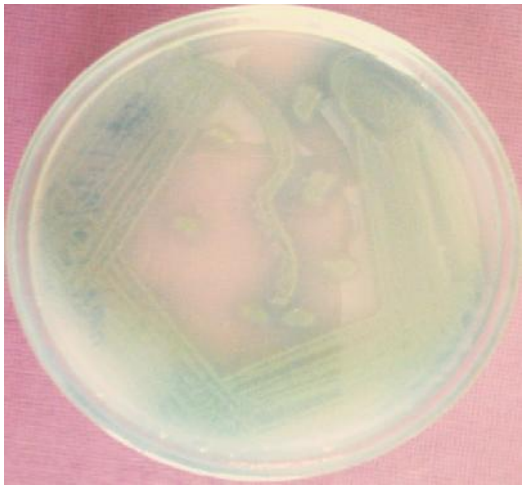


growth on msa medium

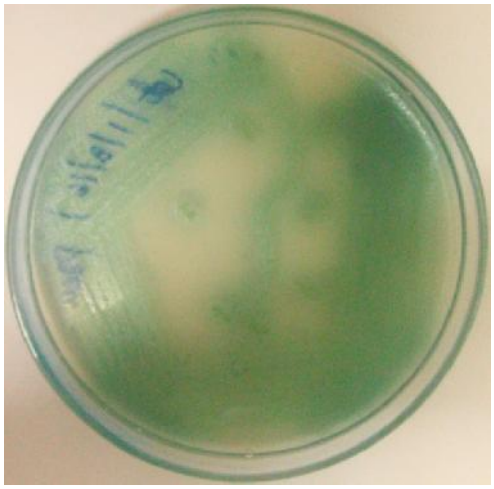


Biochemical test

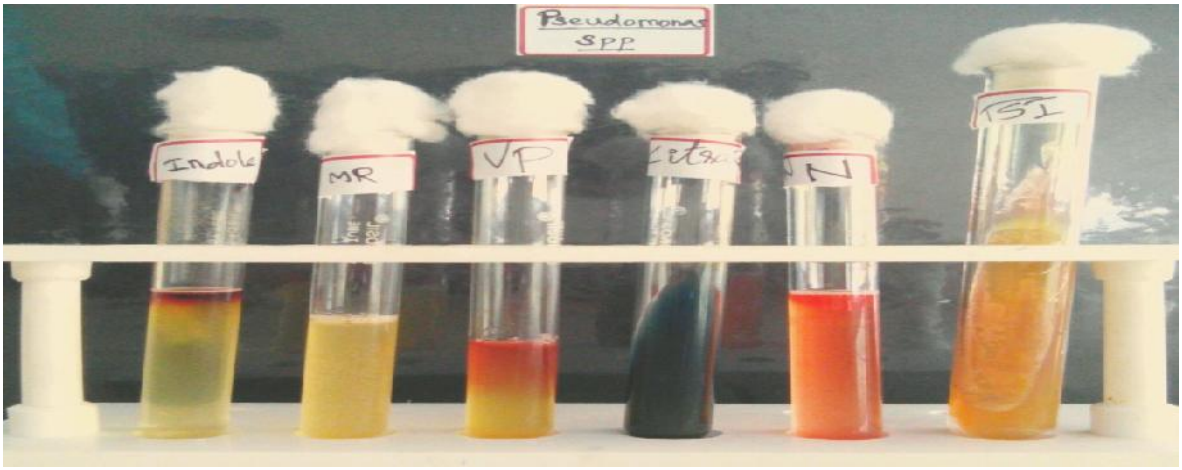
Plate-4isolation and identification of *Pseudomonas aeruginosa*



Growth on nutrient agar



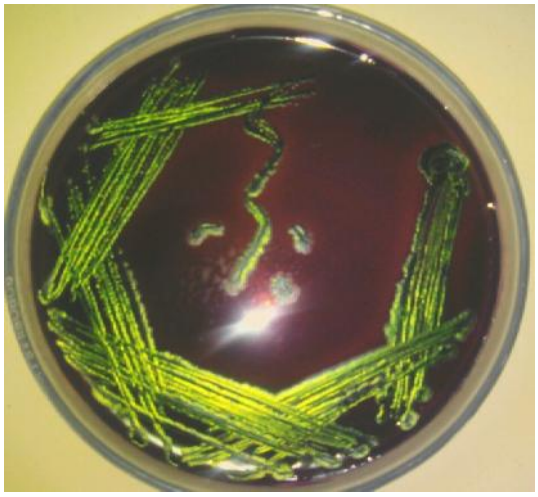
Growth on cetrimide



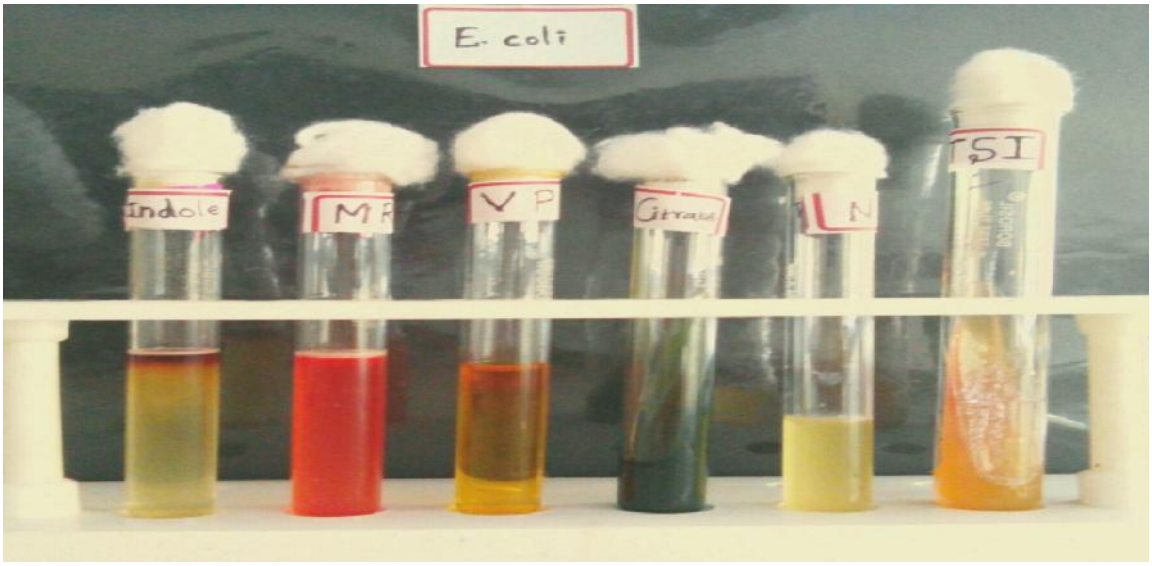
biochemical test

Plate-5 isolation and identification of *E.coli*

GROWTH ON NUTRIENT AGAR



GROWTH ON EMB MEDIUM



Biochemical test

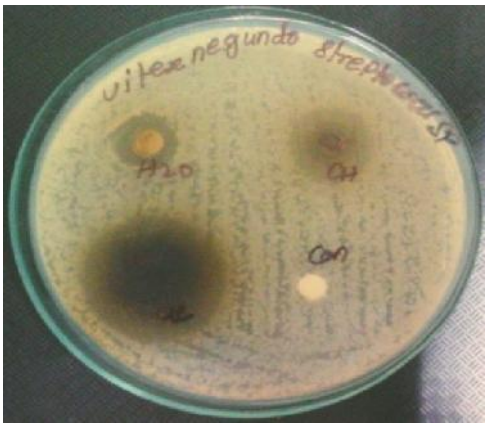
Plate-6 plant extracts

Extracts of *Vitex negundo*

EXTRACTS OF *LEUCAS ASPERA*

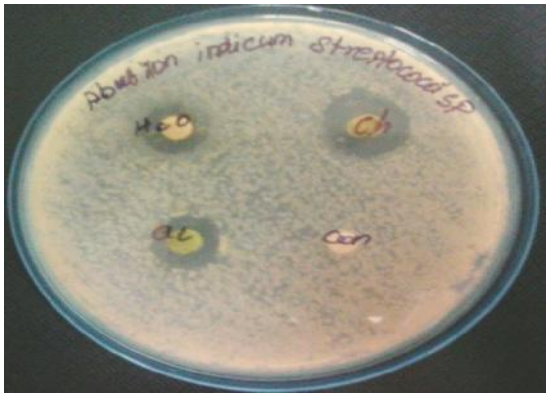
EXTRACTS OF *ABUTILON INDICUM*

PLATE-7 ANTIBACTERIAL ACTIVITY OF EXTRACTS AND FRACTION OF PLANT AGAINST *Streptococcus pyogens*



VITEX NEGUNDO

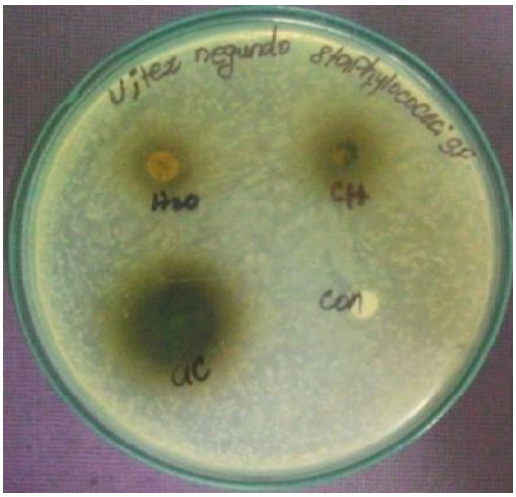
LEUCAS ASPERA



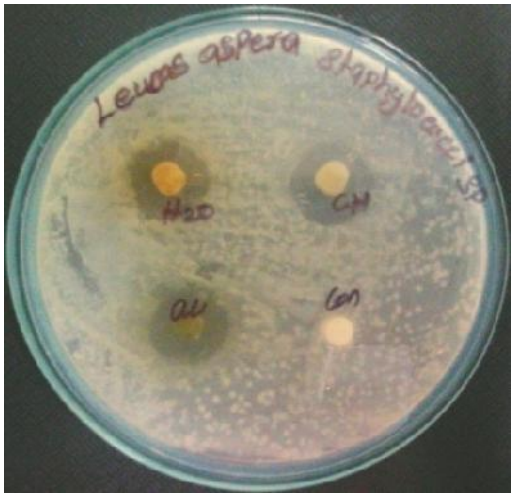
ABUTILON INDICUM

H_2O –WATEREXTRACT AC – ACETONE EXTRACT CH – CHLOROFORM EXTRACTC-CONTROL

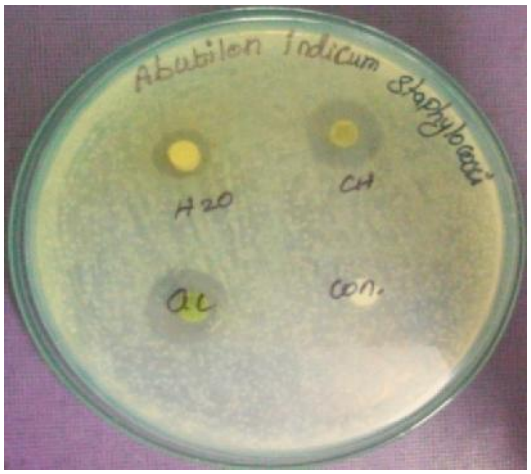
PLATE-8
ANTIBACTERIAL ACTIVITY OF EXTRACTS AND FRACTION OF PLANT AGAINST
Staphylococcus aureus



VITEX NEGUNDO

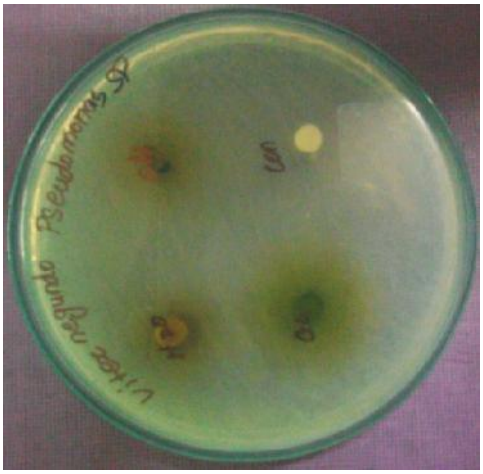


LEUCAS ASPERA

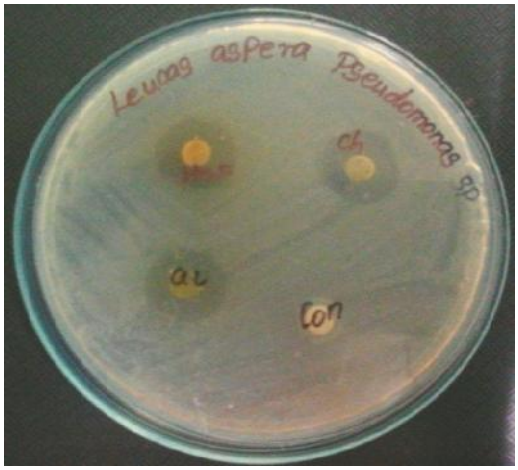


ABUTILON INDICUM
H_2O –WATEREXTRACT
AC – ACETONE EXTRACT
CH – CHLOROFORM EXTRACT
C-CONTROL

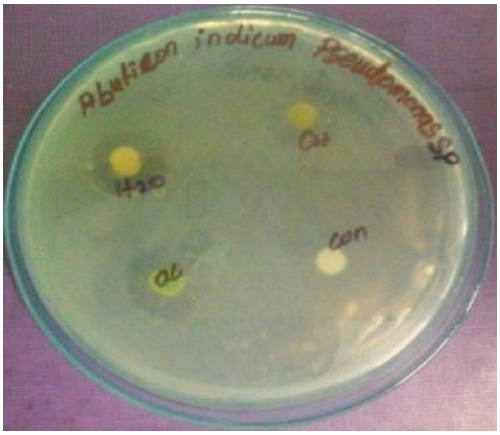
PLATE-9
ANTIBACTERIAL ACTIVITY OF EXTRACTS AND FRACTION OF PLANT AGAINST
Pseudomonas aeruginosa



VITEX NEGUNDO



LEUCAS ASPERA

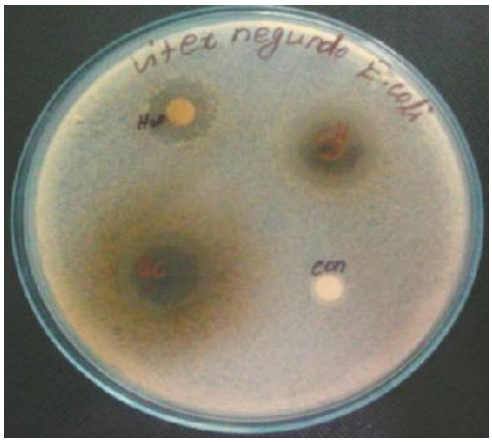


ABUTILON INDICUM

H_2O –WATEREXTRACT
AC – ACETONE EXTRACT
CH – CHLOROFORM EXTRACT
C-CONTROL

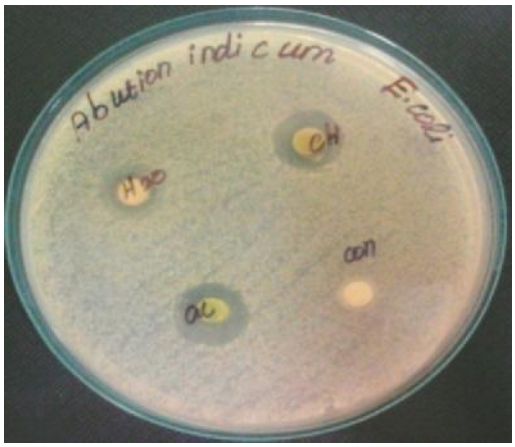
PLATE-10

ANTIBACTERIAL ACTIVITY OF EXTRACTS AND FRACTION OF PLANT AGAINST *E.coli*



VITEX NEGUNDO

LEUCAS ASPERA

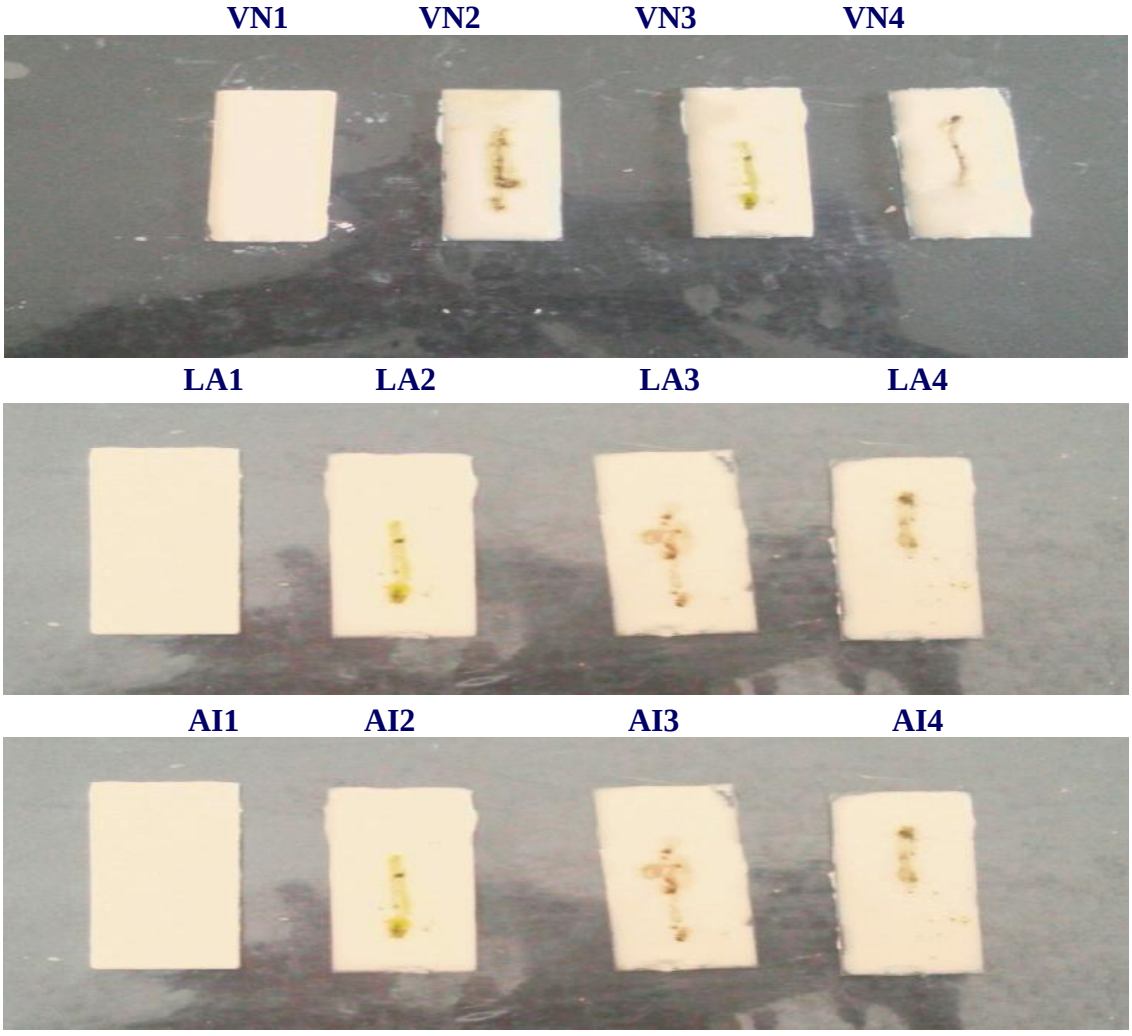


ABUTILON INDICUM

H_2O –WATEREXTRACT
AC – ACETONE EXTRACT
CH – CHLOROFORM EXTRACT
C-CONTROL

PLATE-11

ANALYSIS OF PHYTOCONSTITUENTS OF *VITEX NEGUNDO, LEUCAS ASPERA,ABUTILON INDICUM* USING THIN LAYER CHROMATOGRAPHY



VN1,LA1,AI1 – CONTROL

VN2,LA2,AI2 – WATER EXTRACT

VN1,LA1,AI1 – ACETONE EXTRACT

VN2,LA2,AI2 – CHLOROFORM FRACTION

VN - Vitex negundo

LA – Leucas aspera

AI – Abutilon indicum

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