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ISOLATION AND IDENTIFICCATIION OF BACTERIAL IN INFECTED EYES

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ABSTRACT

High eye swabs were collected from (20) twenty patients (Male and female) eye infection symptoms who attend the eye clinics and government hospital in kumbakonam. During period from January-February 2017. All eye swabs were taken from adult stage patients of these 15 swabs from men, and 15 swabs from women. The age of these patients ranged between, (25-45) years. Then collected samples are transported the microbiology laboratory. The infected eyes sample was serial diluted and inoculated into the nutrient agar medium and incubated. The isolates were gram +cocci, gram - cocci, gram positive rods and gram negative rod shaped and motile organisms. By biochemical characterization, the isolates was identified as belonging to the following genus. The identification of conjuctival isolate samples it shows The amount 20 samples the highest microbial isolate is is *S. aureus* it shows 10 members positive(50%), followed by *s. pyogenes* 8 number of strains mass isolated from infected person 8 (40%). The lower level of microbe's isolates is *E. coli* 2 strains are isolated among 20 persons. The positive result is (10%), keratitis infection samples it shows The amount 20 samples the microbial isolates is *S. aureus* it shows 9 member positive (45%)followed by *K. pnemoniae* 7 number of strains mass isolated from infected person 7(35%). The lower level of microbe's isolates is *E.coli* 4 strains are isolated among 20 persons. The positive result is (10%), The identification of microbes on keratitis infection samples it shows The amount 20 samples the microbial isolates is *s. pyogenes* it shows 11 members positive (55), followed *S. aureus* 8 number of strains mass isolated from infected person 8 (40%). The lower level of microbe's isolates is *E.coli*1 strains are isolated among 20 persons. The positive result is (5%). In the presence study isolated microbes *k. pneumoniae* shows zone of inhibition against antibiotics. Like penicillin, erythromycin, ceftriaxone, ampicillin and tetracycline. The maximum zone of inhibition occurred in penicillin (16mm), ceftriaxone (14 mm), erythromycin (14 mm), tetracycline (12 mm) and the lowest zone of inhibition occurred in ampicillin (5mm). In the present investigations it shows antimicrobial activity of *Lecus aspera* , the isolated microbes *s.aureus* shows maximum zone of inhibition is 25mm,followed by *S. pyogenes* shows zone of inhibition is(23mm),and *k. pneumonia* shows zone of inhibition is (21mm),and the lowest zone of inhibition occurred in *E. coli*. At conclusion the extract from *Lecus aspera* (25mm) and *Ocimum sanctum* (22mm) gives better zone of inhibition compare to chemical antibiotics, and sensitive to bacterial isolates so, patient may use instead of using chemical antibiotics prefer herbal antibiotics in future.

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INTRODUCTION

The eye performs one of the most environment tasks, connecting to the outside world through sight. Even so, it is relatively vulnerable to infections. Our eyes are protected directly or indirectly by active and passive mechanism. Passively, we all have a layer of conjunctivitis and corneal epithelium to prevent the entry of bacteria (Isenberg *et al.,* 2002). The natural tear films contain antimicrobials to fight against any potential harm. Actively, or as a reflex, by blinking our eyes, we promote tear flow washing out pathogens. In addition, our eyes harbor a variety of non - pathogenic bacteria that prohibits pathogenic bacterial growth. However occasionally all these defenses can breakdown, resulting in infection. This could be due to dry eyes, trauma, contact lens wear or immunodeficiency (Madhavan, *et al.,* 2003). Eyelids and conjunctiva harbor a significant number of bacteria and occasionally fungi from the external environment and are called normal flora. (Srivastava et al., .1976). They play an important role in normal body function and health by secreting antibiotics and chemical mediators to maintain surface homeostasis and immune regulation .They also out compete pathogenic bacteria for nutrition thereby inhibiting their growth (Madhavan, *et al.,* 2003).

Conjunctivitis

It is Commonly called "Pink Eye" Inflammation of the conjunctiva Symptoms include: swelling of the conjunctiva and/or eyelids (blepharitis), increased tear production, feeling like a foreign body is in the eye(s) or an urge to rub the eye(s), itching, irritation, and/or burning, discharge (pus or mucus), crusting of eyelids or lashes, especially in the morning, contact lenses that do not stay in place on the eye and/or feel uncomfortable. (Amanda and Harrington, 2016). *Staphylococcus aureus* (*S. aureus*) and *streptococcus pneumonia (S. pneumoniae*) tend to produce oval, yellow white, densely opaque stromal suppuration surrounded by relatively clear cornea.

Keratitis

Symptoms include: eye pain, eye redness, blurred vision, sensitivity to light, excessive tearing, eye discharge, serious condition requiring prompt treatment. May progress s to perforation and blindness if treatment is unsuccessful. Most common infectious cases are bacteria and fungi, followed by parasites and viruses, (Amanda and Harrington, 2016; Van weert, *et al*., 2014). *fusarium, Aspergillus* and *cephalosporium.*

Cataract

A cataract is a clouding of the lens in the eye which leads to a decrease in vision. Cataracts often develop slowly and can affect one or both eyes. Symptoms may include faded colours, blurry vision, and halos around light, trouble with bright lights, and trouble seeing at night. This may result in trouble driving, reading, reading, or recognizing faces (Allan and Vasavada, 2006). Poor vision caused by cataracts may also result in an increased risk of falling and depression. (Cappuccino *et al*., 1992).

Leucas aspera

In addition, uninterrupted use of same herbicides in crop fields makes the weeds resistant against herbicide (Syed and Hyudiuk, 1992; Ejaz Ahmad Javed, *et al.,* 2006) To avoid the detrimental effects of herbicide, there searchers of the different corners of the world are now searching for novel natural plant products to develop bio-herbicides. Numerous weeds and crop plants have been reported to possess allopathic substances in order to compete with neighboring plant species. Active allele chemicals are found in different parts of the plants like leaves, roots, stem, pollens, flowers, fruits and seeds (Syed and Hyudiuk, 1992; Choudhary, 2010). These allelochemicals could be used as lead for herbicide production. Recently, the efforts have been made to identify and isolate these allopathic properties and apply them as a tool for sustainable and eco-friendly weed control strategies (Rao and Rao, 1972).

Ocimum sacntum

Holy basil is most often prepared as a tea or smoked alone or as part of a blend. The leaves and the seeds may both be used. Dried and powdered leaf and fresh leaves are also often consumed, or are mixed with ghee and taken in that way. The essential oil that is extracted from *O. sanctum* is widely used in skin treatments due to its powerful anti-bacterial activity, (Choudhary, 2010) and the dried leaves have even been mixed with stored grain to prevent pest infestation.

MATERIALS METHOD

Site of collection

High eye swabs were collected from (20) twenty patients (Male and female) eye infection symptoms who attend the eye clinics and government hospital in Kumbakonam. During period from January-February 2017. All eye swabs were taken from adult stage patients of these 15 swabs from men, and 15 swabs from women. The age of these patients ranged between, (25-45) years.

Fig 1: Site of collection

Government Hospital Raguram Clinic

High eye infection swabs were taken from patients suffering with redness, itching, endopthalmitis conjunctivitis, keratitis, and cataract. The samples were taken from each patient by using sterile swab sticks, with sterile container. Eye swabs for each patient were transported to the laboratory by inoculating swab into a sterile tube containing 3 ml of normal saline. The samples were examined by staining with gram staining.

All information was taken directly from the patients and special questionaries' sheet was used for each patient, the information included: Patient Name, Data of swabbing age, Location of stay, Educational status, Indoor patients, Symptoms, Diabetes, and Social Status.

The sample were collected by sterile cotton tipped applicator stick in Stuart transport medium. The applicator stick dipped in the eye sample of the patient suffered from keratitis, cataract and conjunctivitis. The cotton dipped eye samples and transported to the laboratory for the further investigation.

Isolation of microorganisms (Sheilds and Sloane, 1991**)**

For isolation of microorganisms, the specimens of eye swab, was directly inoculated culture media; Blood agar, citrimide agar, Nutrient agar, eosin methylene blue agar, Potatoes dextrose agar, macconkey agar, were incubated 37^0 C for 24-48 hours. For the further investigation culture were prepared for Bacterial and Fungal isolation. The indole production test, MR-VP test, vogesproskauer test, citrate utilization test, triple sugar ion agar test, gelatin liquefaction test, starch hydrolysis test, catalase test, urease test, and lipid hydrolysis test were performed to isolation of bacterial on the diseased eyes. The potato dextrose Agar was prepared for isolation of fungal species. The microbial cultures were made inoculated in the tubes and plates using inoculation loops. After, that these tubes were incubated at 27^0 for 48-72 hours.

Collection of plants (Van weert *et al.,* 2014**).**

Fresh plant of ***Lecus aspera*** and ***Ocimum sanctum*** were collected in the morning (6 to 7 am) in the plants were collected from the non-irrigated cultivated lands in and around village of garden at Kumbakonam, Thanjavur (Dt). All the glassware's washed thoroughly in tap water followed by detergent solution and finally rinsed with distilled water. Then were wiped with ethyl alcohol and dried in dust proof cupboard

Sterile of plant materials

The disease free and fresh plants were selected for this investigation. About 2 grams of fresh and healthy leaves were taken for each solvent including aqueous. These are washed with tap water and distilled water for three times. Then, surface sterilized with 0.1 %mercuric chloride or alcohol for few seconds. Again the leaves were washed thoroughly with distilled water (three times)

Preparation of plant extract

The solvent was used for extraction of the leaf extract by cold extraction method. Stalk of the flowers and roots, stems were removed to get leafs alone from both plant. From each leafs both 250 gram of the leafs were soaked in 500 ml of, aqueous, in separate air tight containers. They were allowed to stand at room temperature with occasional homogenize fresh leaves of the container using a sterile pestle and morter. The extracts were separately filtered using sterile whatsmann No. 1 filter paper. The resulted filtrates were then concentrated in a rotary evaporator (Laborator 4000- efficient, Heidolph, Germany) at 400 rpm/50 C. 10 ml of gummy extracts was stored at 4 C for further studies.(Choudhary, 2010; Ejaz Ahmad Javed, *et al.,* 2006)

Fig 2: Preparation of plant extract

Preparation of inoculum

The pure microbial cultures were inoculated into the tubes of nutrient broth, and potato dextrose broth. Using inoculation loops or needles. Then the tubes were incubated at different temperatures and time duration (at 37 C for 24-48 hours for bacteria; and at 28 C for 48-72 hours for fungi). The young cultures were used for antimicrobial susceptibility tests.

Preparation of discs

Whatsmann NO; 1 filter paper was taken and 6mm discs were prepared and sterilized in hot air oven. These discs were loaded with 10/µ of the leaf extract and air dried. This was carried out under sterile condition inside a laminar air flow chamber.

Viable plate count

After incubation, count the colonies on each of the plates. Holding the plate to a light source, Count the colonies by marking their position on the back of the petri plates with a marking pen. This aid in keeping track of those colonies previously counted and avoids recounts. If a plate has more than 300 colonies, reward it as TNTC (too numerous to count). From the plate count data, calculate the concentration of bacteria in the original sample.

For statistical reasons use only data from plates which have between 30 and 300 colonies in this calculations. Each colony forming unit (CFU) represents a single cell or a group of cells attached together and inseparable by shaking. Therefore, the number of cfu in the original sample is determined by multiplying the number of colonies on a dilution plate by the corresponding dilution factor. For example, if there are 200 colonies on the 10^4 plate, then are 200×10,000=2,000,000 colonies or 2×10^6 cfu/ml/in the original sample is. Generally replicates of each dilution are placed, and the mean count is recorded. That the mean of data from all groups in the lab would be an excellent estimate of the number of bacteria in the original sample.

RESULT

In the present study samples collected from conjuctival infection, keratitis and ocular infection was carried out from 20 patients in Kumbakonam in Thanjavur district. Tamil nadu.

Various biochemical test were performed the pathogenic microbes were isolated the result were tabulated below

Table-1 Identification of Microbes on Conjunctival Infection

S.No	Number of trials	Bacterial Isolates	Frequency of occurrence	Percentage%
1	20	*Staphylococcus aures*	10	50
2	20	*Sterptococcus pyogenes*	8	40
3	20	*Escherichia coli*	2	10

The identification of conjuctival isolate sample in (table 1) shows The amount 20 samples the highest microbial isolate is *S. aureus* it shows 10 members positive(50%), followed by *S. pyogenes* 8 number of strains mass isolated from infected person 8 (40%). The lower level of microbe's isolates is *E.coli*2 strains are isolated among 20 persons. The positive result is (10%)

Table-2 Identification of microbes on keratitis infection

S.No	Number of trials	Bacterial Isolates	Frequency of occurrence	Percentage%
1	20	*Staphylococcus aures*	9	45
2	20	*Klebsiella pneumonia*	7	35
3	20	*Escherichia coli*	4	20

The identification of microbes on keratitis infection sample in (table 2) shows The amount 20 samples the microbial isolates is *S. aureus* it shows 9 member positive (45%)followed by *K. pnemoniae* 7 number of strains mass isolated from infected person 7(35%). The lower level of microbe's isolates is *E. coli* 4 strains are isolated among 20 persons. The positive result is (20%)

Table-3 Identification of microbes on ocular infection

S.No	Number of trials	Bacterial Isolates	Frequency of occurrence	Percentage%
1	20	*Sterptococcus pyogenes*	9	45
2	20	*Staphylococcus aures*	7	35
3	20	*Escherichia coli*	4	20

The identification of microbes on keratitis infection sample in (table 3) shows, The amount 20 samples the microbial isolates is *S. pyogenes* it shows 11 members positive (55), followed *S. aureus* 8 number of strains mass isolated from infected person 8 (40%). The lower level of microbe's isolates is *E.coli*1 strains are isolated among 20 persons. The positive result is (5%).

Table-4 Antibiotics Sensitivity

Antibiotics	*E. coli spp*	*Staphylococcus spp*	*Streptococcus spp*	*Klebsiela spp*
Tetracycline	S(15 mm	S(11 mm)	S(12 mm)	S (16mm)
Cefriaxone	S(12mm)	S(10 mm	S(15 mm)	S(14mm)
Ampicillin	S(10 mm)	R	S(5mm)	S(14mm)
Erythromycin	R	S(15 mm)	S(15 mm)	S(12mm)
Penecillin	R	S(18mm)	S(18mm)	S(5)

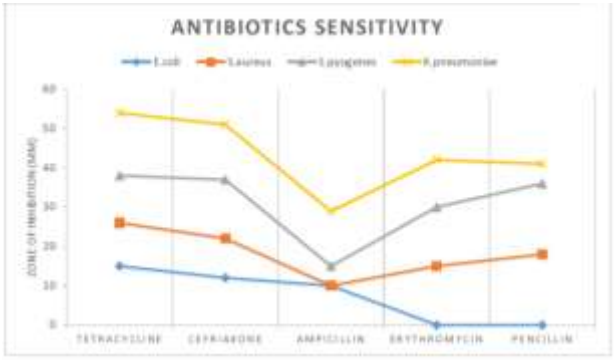

Fig 3-Antibiotics sensitivity

(Table 4) The sensitivity of *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pyogenes* and *Klepsiella pneumoniae* for some types of antibiotics like penicillin, erythromycin, ceftriaxone, ampicillin and tetracycline.

In the present study the isolated microbes *S. aureus* shows zone of inhibition against antibiotics. Like penicillin, erythromycin, ceftriaxone, ampicillin and tetracycline. The maximum zone of inhibition occurred in penicillin (18mm) ,followed by erythromycin (15mm), tetracycline(11mm), cefriaxone (10mm) and the lowest zone of inhibition occurred in cefriaxone (10mm). The ampicillin is only resistant to the *S. aureus*.

In the presence study isolated microbes *S. pyogenes* shows zone of inhibition against

antibiotics. Like penicillin, erythromycin, ceftriaxone, ampicillin and tetracycline. The maximum zone of inhibition occurred in Penicillin

cefriaxone (15mm) ,tetracycline (12mm) and the lowest zone of inhibition occurred in ampicillin (5mm).

In the presence study isolated microbes *E. coli* shows zone of inhibition against antibiotics. Like penicillin, erythromycin, ceftriaxone, ampicillin and tetracycline. The maximum zone of inhibition occurred in tetracycline (15 mm) , (18mm), followed by erythromycin (15mm), followed by ceftriaxone (12mm), and the lowest zone of inhibition occurred in ampicillin (10 mm), erythromycin and Penicillin are resistant to the *E. coli*.

In the presence study isolated microbes *k. pneumonia* shows zone of inhibition against antibiotics. Like penicillin, erythromycin, ceftriaxone, ampicillin and tetracycline. The maximum zone of inhibition occurred in penicillin (16mm), ceftriaxone (14 mm), erythromycin(14 mm), tetracycline (12 mm),and the lowest zone of inhibition occurred in ampicillin (5mm). (Table 4)

Table-5 Morphological and Biochemical characters (Cappuccino *et al.*, 1992)

Morphological and Biochemical character	**Organisms-** ***Streptococcus pyogenes***	**Organisms-** ***Escherichia coli***	**Organisms-** ***Klebsiella pnemoniae***	**Organisms-** ***Staphylococcus aureus***
Morphology	Cocci	Rod	Rod	Cocci
Motility	Non Motile	Motile	Non Motile	Non Motile
Staining	+	-	-	+
Agar slant culture	Thin even growth	White moist Glistening	Slimy white ,translucent raised growth	Abundant opaque Golden growth
Gelatin Liquefaction	-	-	-	+
Starch Hydrolysis	-	-	-	-
H2S Production	-	-	-	-
Indole Production	-	+	-	-
MR Reaction	+	+	-	+
VP Reaction	±	-	±	±
Citrate Reaction	-	-	+	-
Urease Activity	-	-	+	-
Catalase “	+	+	+	+
Oxidase “	-	-	-	-

(Table-5) Shows biochemical characteristics of *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Klebsiella pneumoniae* it shows, gram positive cocci. Non Motile, slant culture appearance like abundant opaque Golden growth. The positive reaction on gelatin liquefaction, methyl red test and, catalase activity, vogesproskauer test on may be positive or negative, and the negative reactions on H2S production, indole production, citrate reaction, urease activity, oxidase activity.

Table-6 Antimicrobial activity of *Leucas aspera* and *ocimum sanctum*

Organisms	antimicrobial activity of *Leucas aspera*	Antimicrobial activity of *Ocimum sacntum*
Staphylococcus sp	S(25mm)	S(22mm)
Streptococcus spp	S(23mm)	S(18mm)
Klebsiella spp	S(21mm)	S(20mm)
E.coli	S(20mm)	S(22mm)

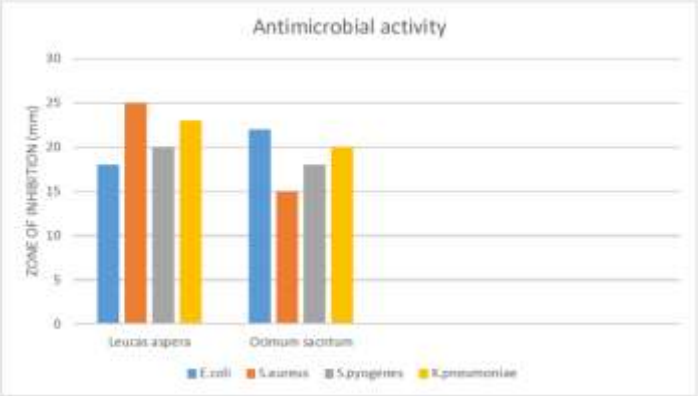

Fig 4: Antimicrobial activity

In the present investigations (Table-6) shows antimicrobial activity of *Leucas aspera* ,the isolated microbes s*taphylococcus aureus shows* maximum zone of inhibition is 25mm,followed by *Streptococcus pyogenes* shows zone of inhibition is(23mm),and *klebsiella pneumonia* shows zone of inhibition is (21mm),and the lowest zone of inhibition occurred in *Escherichia coli.*

In the present investigations (Table-6) shows antimicrobial activity of *Ocimum sanctum*, the isolated microbes *staphylococcus aureus* and *Escherichia coli* shows maximum zone of inhibition is (22mm),followed by *klebsiella pneumonia* shows zone of inhibition is (20mm),and the lowest zone of inhibition occurred in *Streptococcus pyogenes* (18mm).

The sensitivity of *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pyogenes* and *Klepsiella pneumoniae* for *Lecus aspera* and *Ocimum sanctum.*

DISCUSSION

In this present study an attempt is made to isolate and identify the conjuctival isolates from the cataract patient cojuctivities and keratitis. The prevalence of coagulase Negative *Staphylococci* was similar to the studies conducted by Isenberg et al., (2002). Other isolates were *Staphylococcus aureus* and *E. coli*, *Streptococcus pyogenes*, *Klebsiella pnemoniae*, isolated and anti biograms was made in Herbal plant leaf extracts of *Ocimum sanctum* and *Leucas aspera* and also commercial antibiotics Amphot-cin-B, Cefriaxone,Penicillin-G, Tetracycline, Ampicillin, ,Erythromycin.

In *ocimum sacntum* is a fragrant bushy perennial growing up to 1.5m in hight with profusion of white blooms and slightly purple tinted foliage. *Leucas aspera* is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiole, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long; flowers white, sessile small, in dense terminal or axillary whorls; bracts 6 mm long, linear, acute, bristle; tipped, ciliate with long slender hairs;calyxvariable,tubular,8-13 mm long

Leaf extract were used for antimicrobial activity of isolated microorganisms like *Staphylococcus aureus* and *E. coli, Streptococcus pyogenes, Klebsiella pnemoniae*, the isolates were identified on the basis of their cultural and biochemical characteristics according to bergeys's manual of deteminative bacteriology (9th edition) an profiles(Syed and Hyudiuk, 1992) Phenotypic examination of the recovered microorganisms revealed that they belong to the genera of *Staphylococcus aureus* and *Escherichia coli, Streptococcus pyogenes, Klebsiella pnemoniae*. All selected strains showed optimal growth at

300C.thesefindings were in agreement with a Study by (Sheilds and Sloane, 1991; Rao PN and Rao, 1972).

Endophthalmitis means bacterial or fungal infection inside the eye involving the vitreous and/or aqueous humors. Most cases are exogenous and occur after eye surgery, after penetrating ocular trauma, or as an extension of corneal infection. An increasing number of cases are occurring after intra virtual injections of anti-vascular endothelial growth factor (VEGF) medications. Endophthalmitis may also be endogenous, arising from bacteramic or fungaemic seeding of the eye. The infected eye never serves as a source of bacteremia or fungaemia, however. The most common pathogens in endophthalmitis vary by category. Coagulase –negative staphylococci are the most common cause of post-cataract endophthalmitis. In this present study an attempt is made to isolate and identify the conjucntival isolates from the cataract patients and their anti biogram using standard antibiotics was carried out. The prevalence of CONS Was Similar to the studies conducted by (Syed and Hyudiuk, 1992).

In our finding the frequency of occurrence of microbes higher in conjuctival isolates are *Staphylococcus aureus* (50%), followed by *Streptococcus pyogenes* (40%), and lowest *Escherichia coli* (10%). The infection conjunctivitis, defined inflammation of the bulbar, mucous membrane that covers both the surface of the eye and the lining of the undersurface of the eyelid in United States are related to conjunctivitis affecting about 6 million people annually

Sudhakar *et al.,* (2006) only about 30% of primary care parents, with infectious conjunctivitis are confirmed to have bacterial conjunctivitis, although 80% are treated with antibiotics. (Voogelbreinder *et al.,* 2009). The maximum zone of inhibition is (22mm) *staphylococcus aureus* in *Ocimum sanctum*. This work was supported by Prescott *et al.,* (2002).Under certain circumstances, topical antibiotics for treating bacterial antibiotics (Levi, 1981).

Microbes isolated from keratitis infected patients are *staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae*. The occurrence of *staphylococcus aureus* predominant in all the three infection, followed by *Escherichia coli, Streptococcus pyogenes, Klebsiella pnemoniae.*

In the present investigation the antibiotic resistant test the *Staphylococcus aureus*, shows the maximum zone of inhibition occurred in penicillin (18mm), followed *Streptococcus pyogenes*, shows the maximum zone of inhibition occurred in tetracycline(15 mm),*Escherichia coli,* the maximum zone of inhibition occurred in tetracycline(15 mm), and *klebsiella pneumonia* the maximum zone of inhibition occurred in penicillin (16mm).

The plant leaf extract from *Leucas aspera* shows maximum zone of inhibition is (25mm) in *Staphylococcus aureus***.** The plant leaf extract from *Ocimum Sanctum* shows maximum zone of inhibition is (22mm) in *Staphylococcus aureus* and *Escherichia coli*.

At conclusion the extract from *Leucas aspera* (25mm) and *Ocimum sanctum* (22mm) gives better zone of inhibition compare to chemical antibiotics, and sensitive to bacterial isolates so, patient may use instead of using chemical antibiotics prefer herbal antibiotics in future.

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