ANALYSING ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL STUDY OF MICROBES FROMOPHTHALMIC INFECTION AGAINST *OCIMUM SACNTUM. L, LECUS ASPERA. L, TABERNAEMONTANA DIVARICATA. L, RICINUS COMMUNIS. L.*

R.Sabina gowri[1], Dr.R.Krishnaveni[1], Dr.V.Eugin Amala[1]
[1]PG and Research Department of Microbiology, Idhaya College for Women,
Kumbakonam – 612 001

Abstract: The infected eye swabs were collected from 20 patients (Male and female) eye infection symptoms who attend the eye clinics and government hospital in kumbakonam. During period from January-February 2018. 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 + rods and gram -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%), The lower level of microbes 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%) The lower level of microbes 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), The lower level of microbes isolates is *E.coli* 1 strains are isolated among 20 persons. The positive result is (5%).In the present investigation the antibiotic resistant test the *staphylococcus aureus,* shows the maximum zone of inhibition occurred in Erythromycin (12mm), followed *Streptococcus pyogenes,* shows the maximum zone of inhibition occurred in penicillin (14mm), *Escherichia coli,* Ampicillin (12 mm), and *Pseudomonas aeruginosa,* Ampicillin and Erythromycin (12mm). and *Proteus sp* - tetracycline and Erythromycin (12 mm). In the present investigations it shows antimicrobial activity of Medicinal plants, The maximum zone of inhibition is (15mm) *staphylococcus aureus* in *Tabernaemontana Divaricata* (Acetone with ethanol extract) (17mm), *Streptococcus pyogenes*in *Ocimum sanctum* (Acetone with ethanol extract) (20mm), *Escherichia coli* in *Ocimum sanctum* (Aceton extract), (15mm) *Pseudomonas aeruginosa*in *Lecus aspera* (Ethanol extract) and (15mm) *Proteus sp* in *Ricinus communis*. Phytochemical screening of individual extracts were qualitatively tested for the presence of various phytochemical constituents namely alkaloids, carboxylic acids, coumarins, flavonoids, phenols, quinones, resins, steroids, fixed oils, saponins, tannins, glycosides In the present investigation the HPLC technique test give some peaks in each particular herbal plant leaf extract , the plants are *Leucas aspera, Ocimum sanctum ,Ricinus communis, Tabernaemontana divaricata* that result given some phytochemicals components like, Hydroxybenzonic acid, Luteolin, Benzoic acid, Coumeric acid, Rutin, Hdroxy benzoic acid, Gallic acid, Ellagic acid, Ocimene, Germacrene, Coumaric acid, Syringic acid, Ferulic acid, Chlorogenic acid, Catechin, Ethyllinoleate, Hexahydroxyformesyl acetone, Mysristic acid, Apigenin, Quercetin are present in given plant extract. At conclusiongives 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.

***Index terms : Tabernaemontana divaricate, Ocimum sanctum, Lecus aspera, Ricinus communis,* HPLC, eye infection, *E.coli, S.pyogenes, P.aeruginosa, S.aureus, Proteus sp,* Phtochemical, Antimicrobial, Antibiotic**

1. INTRODUCTION

The Eye is the organ of sight. Eyes enable people to perform daily tasks and to learn about the world that surrounds them. Sight, or vision, is a rapidly occurring process that involves continuous interaction between the eye, the nervous system, and the brain. When someone looks at an object, The most important causes of bacterial eye infections are :*Pseudomonas aeroginosa , Neisseria gonorrhoea, Proteus spp* , such *Moraxella lacunata, lacunat, Staphylococcus aureus, Streptococcus pyogenes* (17)(19) All parts of the eye (4) may be infected by bacteria, fungi, parasites, or viruses. Anti-infectives such as antibiotics(ATB), antiseptics, antifungals, anti-helminths or antivirals can be used depending on the type of infection (2). Conjunctivitis 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 (1). Keratitis symptoms include: eye pain, eye redness, blurred vision, sensitivity to light, excessive tearing, eye discharge, Most common infectious cases are bacteria and fungi, followed by parasites and viruses (1). A cataract is a clouding of the lens in the eye which leads to a decrease in vision. 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. The phytochemical screening study was used to discover the medicinal benefits and the nutrients and dietary fiber present in the plant that can protect against diseases (20)(21). In this experiment the presence of alkaloids, flavanoids, saponins, steroids, triterpenoids, glycosides, carbohydrate, vitamin C, proteins and free amino acids was tested by simple qualitative methods (18). *O.sanctum* the objective of the present work was to make an analysis of the ethno botanical information on the medicinal plants used in diabetes mellitus control, and of the results obtained in the investigation of the hypoglycemic Activity of such plants (22). anti-hyperglycemic and hypolipidemic activities of this in normal, glucose induced (13). The aim of the present work was to evaluate the antioxidant potential of *L. aspera* extracts using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging capacity, *L. aspera* to further establish the scientific basis of the traditional uses of the plant (23). *T. divaricata* are well recognized, its role in antimicrobial therapy is not yet well studied. Therefore, the *Tabernaemontana divaricata* against the selected bacterial strains. The present day taxonomic description of the flowers of *T. divaricata* is based on the traditional taxonomic principles. *Ricinus communis* is major portion is present in leaves, root and seeds. *Ricinus communis* seeds oil is a valuable triglyceride fatty acid commonly called as vegetable oil got through the crushing and then mechanically pressing of seeds of the *Ricinus communis* (7). Let talking about its composition.

2. MATERIALS METHOD

2.1. 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 2018 (3). The specimens of eye swab, was directly inoculated culture media; were incubated For The biochemical tests were performed to isolation of bacterial on the diseased eyes.

2.2. COLLECTION OF PLANTS AND AUTHENTICATION

Communiant of *Leucas aspera* and *Ocimum sanctum, Ricinus communis* and *Tabernaemontana divaricata* were collected in the morning (6 to 7 am) in a The plants were collected from the non-irrigated cultivated lands in and around village of garden at kumilankuzhi, Ariyalur (Dt). The samples were identified and authenticated by Dr.John Britto, Rabinet Herbarium, st. joseph's college, Trichy, Tamilnadu, India and given the Voucher specimen No. RSG/001/2018, RSG/002/2018, RSG/003/2018, RSG/004/2018 respectively

2.3. PREPARATION OF PLANT EXTRACT (24)

The solvent was used for extraction of the leaf extract by cold extraction method. From each leafs both 250 gram of the leafs were soaked in 500 ml of aqueous, Ethanol and acetone in separate air tight containers. with occasional homogenize fresh leaves of the container using a sterile pestle and mortar. filtered using sterile whatsmann No. 1 filter paper.

2.4. PREPARATION OF INOCULUM (25) (26)

The pure microbial cultures were inoculated into the tubes of nutrient broth, and potato dextrose broth. 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 test.

2.5. ANTIMICROBIAL ACTIVITY (27)

On the present investigation the plants *Leucas aspera, Ocimum sanctum, Tabernaemontana divaricata, Ricinus communis.* used for antibacterial activity.

2.6. PHYTOCHEMICAL ANALYSIS:

Phytochemical screening of individual extracts were qualitatively tested for the presence of various phytochemical constituents namely alkaloids, carboxylic acids, coumarins, flavonoids, phenols, quinones, resins, steroids, fixed oils, saponins, tannins, glycosides according to the tests (18).

2.7. HPLC TECHNIQUE METHOD

Quantitative analysis of the sample was performed according to the method of (14), at st. joseph's college, Trichy, Tamilnadu, India. The peak area was calculated with a Winchrom integrator. The plants are *Leucas aspera* and *ocimum sanctum, Tabernaemontana divaricata, Ricinus communis.* Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column by using mobile phases are Methanol and Acetonitrile, and added Triflora acetic acid (290 nm, 278 nm, 254 nm, 254 nm, particle size 2 µm, 5µ C-18 at 25°C. Running conditions included: tannins and phenolic compounds and flavonoids, elagic acid etc. (5).

3. RESULTS (10)

TABLE-1- ANTIBIOTICS SENSITIVITY ASSAY

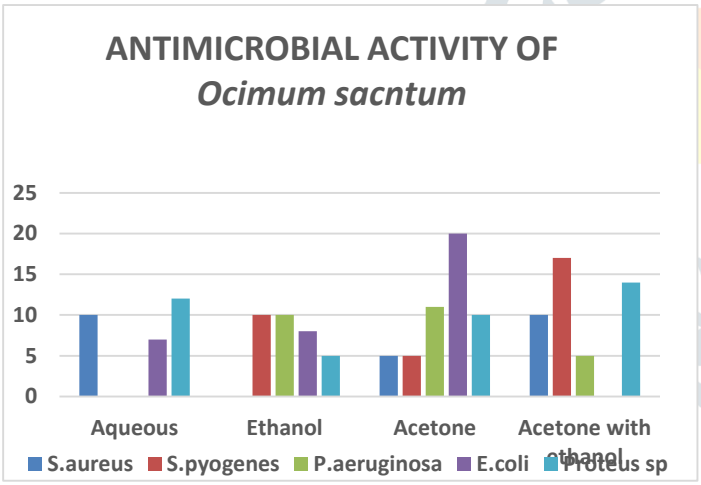
ANTIBIOTICS	*E.COLI*	*S.AUREUS*	*S.PYOGENE*	*P.AERUGINOSA*	*PROTEASE SP*
Tetracycline	S(10 mm	S(6mm)	S(11 mm)	S (12mm)	S (12mm)
Cefriaxone	S(5mm)	S(10 mm	R	S(10mm)	R
Ampicillin	S(12 mm)	R	S(6mm)	S(12mm)	S(5mm)
Erythromycin	R	S(12 mm)	R	S(12mm)	S(12mm)
Penecillin	R	S(11mm)	S(14mm)	S(5)	R

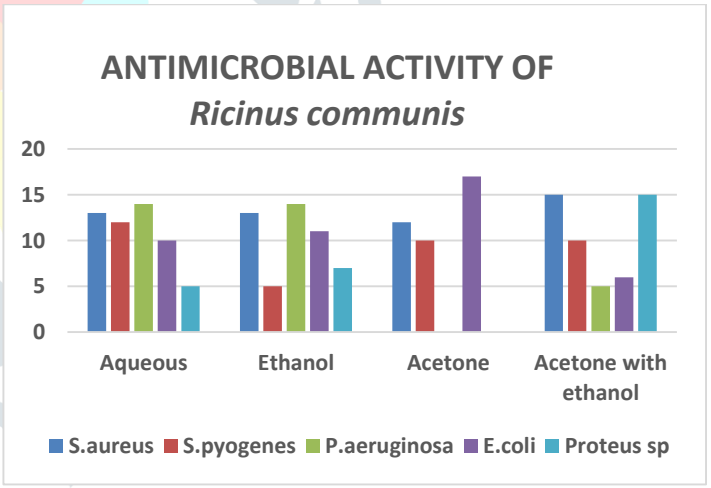
In the present study the isolated microbes *staphylococcus aureus* shows zone of inhibition against antibiotics. L The maximum zone of inhibition occurred in erythromycin (12 mm), the lowest zone of inhibition occurred in cefriaxone (10 mm). *Streptococcus pyogenes* shows The maximum zone of inhibition occurred in Penicillin (14 mm), ampicillin (6 mm). *Escherichia coli* shows . The maximum zone of inhibition occured inin ampicillin (12 mm) and the lowest zone of inhibition occurred in ceftriaxone (5 mm), *Proteus sp* shows zone of inhibition against antibiotics. The maximum and minimum zone of inhibition occurred in erythromycin (14 mm), and ampicillin (5 mm), *Pseudomonas aeruginosa* shows The maximum and minimum zone of inhibition occurred in ampicillin, erythromycin, and tetracycline (12 mm) and Penicillin (5 mm).

PHYTOCHEMICAL SCREENING TEST

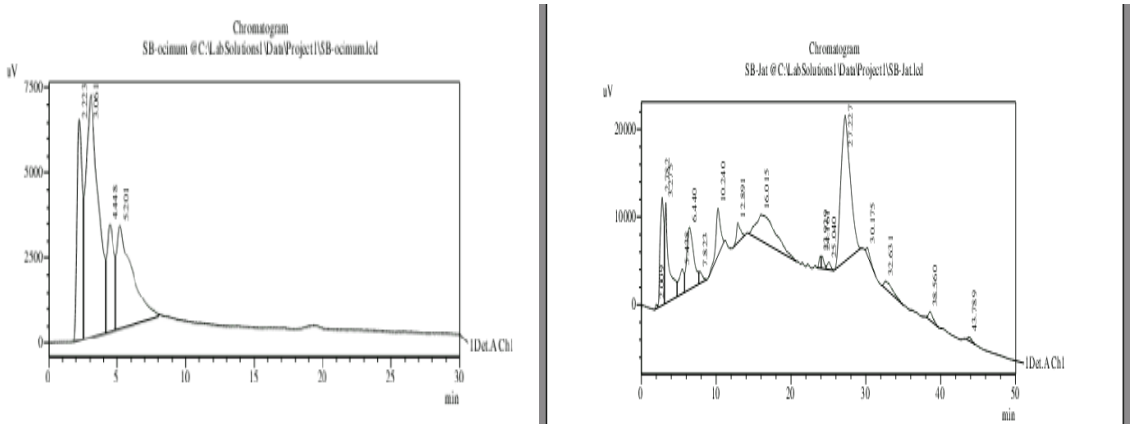
Ricinus communis - Alkaloids(+), Terpenoids(+), Cardiac glycosides(+), Tannis(+), Flavonoids(-), Steroids(+), Saponins(+), Anthraquinone(-), Reducing sugar(-), Aminoacids(+).

cimum sanctum - Alkaloids(+), Flavonoids(+), Steroids(+), Tannis(+), Terpenoids(+), Glycosides(+), CarbohydrateVitamin c(+), Proteins(+), Aminoacid(+).





In the present investigations shows antimicrobial activity of the maximum zone of inhibiton occurred in *Escherichia coli* (20mm) in *Ocimum sanctum*, (Acetone extract) and minimum zone *of* inhibition occurred in *staphylococcus aureus, S.Pyogenes, Proteus sp* (5mm) in *Leucas aspera and ocimum sanctum* extract and *Escherichia coli* (5mm), *P.aeruginosa* in *ricinus communis* acetone with ethanol extract.



HPLC 'fingerprints' of the extracts of aqueous extracts of*Leucas aspera*, showed major peaks (more concentration of components) at the retention times (min.) of,13.11014.725, 15.677, 18.238, 21.125 respectively at wavelength of 290 nm aqueous extracts of *Ocimum sanctum* -2.223, 3.061, 4.448, 5.201, respectively at wavelength of 278 nm and *Tabernaemontana divaricata* -20.245, 17.830, 17.475, 12.912, 10.995 respectively at wavelength of 254 nm. *Ricinus communis* - 43.789, 38.560, 32.631, 30.175, 27.227, 25.040, 24.161 respectively at wavelength of 254 nm.

DISSCUSSION

In this present study an attempt is made to isolate and identify the conjuctival isolates from the cataract patient conjunctivitis and keratitis. The prevalence of coagulase Negative *Staphylococci* was similar to the studies conducted by (16). Other isolates were *Staphylococcus aureus* and *Escherichia.coli, Streptococcus pyogenes, Pseudomonas aeruginosa , Proteus sp* isolated *and* antibiograms was made in Herbal plant leaf extracts of *Ocimum sanctum and Leucas aspera, Tabernaemontana divaricata, Ricinus communis* and also commercial antibiotics Amphot-cin-B, Cefriaxone, Penicillin-G, Tetracycline, Ampicillin, Erythromycin.

Leaf extract were used for antimicrobial activity of isolated microorganisms like *Staphylococcus aureus* and *E.coli , Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus sp* 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 Phenotypic examination of the recoverd microorganisms revealed that they belong to the genera of *Staphylococcus aureus* and *Escherichia coli , Streptococcus pyogenes, Pseudomonas aeruginosa, Proteus sp* . All selected strains showed optimal growth at 30^0 C.these findings were in agreement with a Study by (11).(17)

Phenolic profile was performed using an Agilent 1100 model HPLC with automatic injection wavelength UV/VIS detector, and acquisition system (Agilent Software 1100, Santa Clara, Calif., U.S.A.). Chromatographic separations were performed on a C_{18} reverse phase column LiChroCART (25 x 0.4 cm, particle size 5 mm), using water/formic acid (99:1) (A) and acetonitrile (B) as mobile phase at 1 mL min^{-1} and samples of 10 microliters. Gradient was applied, starting with 2 % B in A, uploading to 32 % B at 30 min, isocratic for 18 min, up to 55 % B at 48 min, 95 % B in A for 53 min, isocratic for 4 min and returning to initial conditions (2 % B) to 65 min. The resolved compounds were detected at 280 nm, identified, and quantified on the basis of chromatographic retention times of coeluted pure standards. Eight pure commercial phenolic acids (gallic, vanillic, chlorogenic, caffeic, ellagic, rosmarinic, coumaric, and ferulic acids), catechin, epicatechin, epigallocatechin gallate, rutin, quercetin, kaempferol, naringin, hesperidin, and umbelliferone were used for calibration and quantification (Sigma, St Louis, MO, USA).Tapan (12)

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 (14) Only about 30% of primary care parents, with infectious conjunctivitis are confirmed to have bacterial conjunctivitis, although 80% are treated with antibiotics.

Microbes isolated from keratitis infected patients are *staphylococcus aureus, Escherichia coli,*. The occurrence of *Pseudomonas aeruginosa , staphylococcus aureus,* predominantion all the three infection, followed by *Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa* and *Proteus sp.*

In the present investigation the antibiotic resistant test the *staphylococcus aureus*, shows the maximum zone of inhibition occurred in Erythromycin (12mm), followed *Streptococcus pyogenes*, shows the maximum zone of inhibition occurred in penicillin(14mm), *Escherichia coli* the maximum zone of inhibition occurred in Ampicillin (12 mm) and *Pseudomonas*

aeruginosa the maximum zone of inhibition occurred in Ampicillin and Erythromycin (12mm). and *Proteus sp* the maximum zone of inhibition occurred in tetracycline and Erythromycin (12mm).

In the present investigations it shows antimicrobial activity of Medicinal plants. The maximum zone of inhibition is (15mm) *staphylococcus aureus* in *Tabernaemontana divaricata* (Acetone with ethanol extract), (17mm)*Streptococcus pyogenes* in *Ocimum sanctum* (Acetone with ethanol extract) ,(20mm) *Escherichia coli* in *Ocimum sanctum*(Acetoneextract), (15mm) *Pseudomonas aeruginosa* in *Lecus aspera* (Ethanol extract) and (15mm) *Proteus sp* in *Ricinus communis* . This work was supported by (8). Under certain circumstances, topical antibiotics for treating bacterial antibiotics (6).

In the present investigation the HPLC technique test give some peaks in each particular herbal plant leaf extract, the plants are *Leucas aspera, Ocimum sanctum, Ricinus communis, Tabernaemontana divaricata* that result given some phytochemicals components like, Hydroxybenzonic acid, Luteolin, Benzoic acid, Coumeric acid, Rutin, Hdroxy benzoic acid, Gallic acid, Ellagic acid, Ocimene, Germacrene, Coumaric acid, Syringic acid, Ferulic acid, Chlorogenic acid, Catechin, Ethyllinoleate, Hexahydroxyformesyl acetone, Mysristic acid, Apigenin, Quercetin are present in given plant extract.

At conclusion 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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