



CODEN [USA]: IAJ PBB

ISSN : 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**

SJIF Impact Factor: 7.187

<https://doi.org/10.5281/zenodo.23279097>Available online at: <http://www.iajps.com>

Research Article

**ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL
COMPONENTS OF LEAF EXTRACT OF *PINUS LONGIFOLIA*****Bhimanani Rishika*, S. Sunayana**Department Of Pharmacology, Princeton College Of Pharmacy In Pocharam ,Hyderabad,
Ghatkesar, Telangana.**Abstract:**

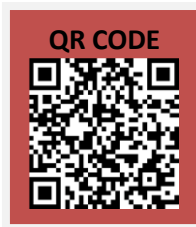
The technique improves patient compliance by reducing both dose and the frequency of administration and thus minimizing the local as well as systemic toxic effects. The aim of the present research work was to of Methanolic extracts of Nymphaea stellata in the doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg were used in Wistar rats of either sex. Were measured by standard biochemical methods. Silymarin (500 mg/kg) was used as a standard drug for assessment of Anti-Microbial status. When compared with the standard Anti-Microbial agent Ciprofloxacin, The antibacterial activity of these prepared nanoparticles against pathogenic bacterium has shown.

The anti-inflammatory activity was determined by carrageenan-induced paw edema rat. The results demonstrated that Nymphaea stellata is effective for both acute and chronic inflammation. Thus, these results have suggested that Nymphaea stellata possesses anti-inflammatory activities.

keywords: - Anti-Microbial agent, anti-inflammatory activity, Nymphaea.

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Please cite this article in press **Bhimanani Rishika et al., Antibacterial Activity And Phytochemical Components Of Leaf Extract Of Pinus Longifolia., Indo Am. J. P. Sci, 2026; 13(10).**

INTRODUCTION:**INTRODUCTION OF ANTI- MICROBIAL ACTIVITY**

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization ^{1, 2}. They usually contain many biologically active ingredients and are used primarily for higher treating mild or chronic ailments. According to World Health Organization (WHO), about 80% of the world population relies chiefly on the plant based traditional medicine especially for their primary healthcare needs. Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, safety margins and lesser costs ^{3, 4}.

Infectious diseases are a major cause of morbidity and mortality worldwide. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidemics of human immunodeficiency virus (HIV) infections ^{5, 6}. This situation provided the impetus to the search for new antimicrobial substances from various sources like medicinal plants. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects. Therefore, there is a need to search for new infection-fighting strategies to control microbial infections ⁷.

Plant medicines are used on a worldwide scale to prevent and treat infectious diseases ⁸. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, terpenoids and flavonoids having been found in vitro since they have antimicrobial properties and may serve as an alternative, effective, cheap and safe antimicrobial for the treatment of microbial infections ⁹. Plant based antimicrobial compounds have great therapeutic potential as they have lesser side effects as compared with synthetic drugs and also little chance of development of resistance. Therefore an attempt has been made to study the antibacterial activity of ten medicinally important plants viz. *Artocarpus heterophyllus*, *Berberis aristata*, *Chromolaena odorata*, *Embelia ribes*, *Jasminum angustifolia*, *Mahonia leschenaultii*, *Pluchea lanceolata*, *Plumbago indica*, *Terminalia chebula*, *Vitex negundo*.¹⁰

INTRODUCTION OF BACTERIA

Clinical isolated bacteria used in the study are *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Escherichia coli**Classification**

Escherichia coli is the most commonly encountered member of the family Enterobacteriaceae in the normal colonic flora and the most common cause of opportunistic infections. All members of the family Enterobacteriaceae are facultative, all ferment glucose and reduce nitrates to nitrites and all are oxidase negative.¹¹

Domain	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	<i>Escherichia</i>
Species	<i>coli</i>

Table-1 Classification of *E. coli*



Figure 1.7:Gram stain of *E. coli*.

Morphology and identification

Escherichia coli is gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae. Because most strains rapidly ferment lactose, colonies grown on MacConkey media are smooth, glossy, and translucent and are rose-pink in colour. Some strains grown on blood agar result in colonies being surrounded by zones of haemolysis. Colonies are smooth, circular, 1 – 1.5mm in diameter and yellow opaque if lactose fermenting (blue, if non-lactose fermenting) when grown on cystine-lactose-electrolyte deficient (CLED) medium.¹²

Epidemiology

Strains of *Escherichia coli* predominate among the aerobic commensal bacteria present in the healthy gut.¹³

Escherichia coli Infections

Escherichia coli was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including meningeal, gastrointestinal,

urinary tract, wound and bacteremia infections in all age groups.¹⁴

Other infections caused by *Escherichia coli* include peritonitis, cholecystitis, septic wounds and bedsores. They may also infect the lower respiratory passages or cause bacteraemia and endotoxic shock especially in surgical or debilitated patients.¹⁵

Antimicrobial Susceptibility

Within the community, *Escherichia coli* strains are commonly susceptible to all agents active against the Enterobacteriaceae. However, because of the frequent occurrence of R plasmids, strains acquired in hospitals may be resistant to any combination of potentially effective antimicrobics and therapy must therefore be guided by susceptibility testing.¹⁶

Staphylococcus aureus

Classification

Members of the genus *Staphylococcus* (staphylococci) are Gram-positive cocci that tend to be arranged in grape-like clusters.¹⁷

Table-2 Classification of *S. aureus*

Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Staphylococcaceae
Genus	<i>Staphylococcus</i>
Species	<i>aureus</i>

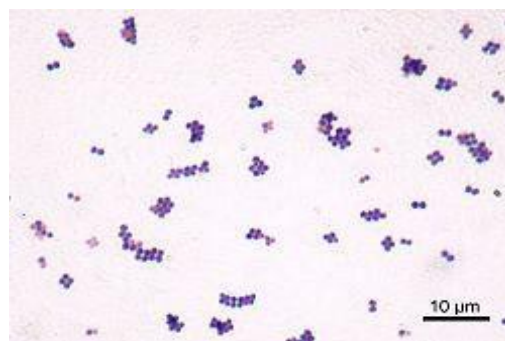


Figure 1.8: Gram stain of *S. aureus*

Morphology and identification

Staphylococci are spherical cells about 1 μm in diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form spores.

Staphylococcus aureus is a facultative anaerobe that grows at an optimum temperature of 37°C and an optimum pH of 7.5.

S. aureus produces white colonies that tend to turn a buff-golden color with time, which is the basis of the species epithet aureus (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony.

On nutrient agar, following aerobic incubation for 24 hours at 37°C, colonies are 1 – 3mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On

MacConkey agar, colonies are small to medium in size and pink or pink-orange in colour.

Epidemiology

Staphylococci are highly successful colonizers of humans and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occur worldwide, and newly emerging hypervirulent or multiresistant strains spread rapidly over wide geographical areas. The bacteria survive in the air, on objects or in dust for days, therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources.¹⁸

Infections

S. aureus causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood. include

Domain Bacteria

Phylum Proteobacteria

Class Gammaproteobacteria

Order Pseudomonadales

Family Pseudomonadaceae

pneumonia, bacteremia leading to secondary pneumonia and endocarditis, osteomyelitis secondary to bacteremia and septic arthritis, seen in children and in patients with a history of rheumatoid arthritis. Diseases caused by Staphylococcal toxins include scalded skin syndrome and toxic shock syndrome.¹⁹

Antimicrobial Susceptibility

Resistance to penicillin G can be predicted by a positive test for β -lactamase; approximately 90% of *S. aureus* produce β -lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 35% of *S. aureus* and approximately 75% of *S. epidermidis* isolates.

Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics.²⁰

Pseudomonas aeruginosa

Classification

Pseudomonas aeruginosa is a classic opportunist pathogen belonging to the genus *Pseudomonas*.



Figure 1.9: Gram stain of *P. aeruginosa* cells

Morphology and Identification

Is obligate aerobe, motile, rod-shaped, measuring about 0.6 x 2 μ m. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains. sometimes producing a sweet or grape-like or corn taco-like odor.²¹

Its production of blue, yellow, or rust-colored pigments differentiates it from most other Gram-

negative bacteria. The blue pigment, **pyocyanin**, is produced only by *P.*

aeruginosa. **Fluorescein**, a yellow pigment that fluoresces under ultraviolet light, is by *P. aeruginosa* and other free-living less pathogenic *Pseudomonas* species. Pyocyanin produced and fluorescein combined produce a bright green color that diffuses throughout the medium.²²

P. aeruginosa grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other *Pseudomonas* species. It does not ferment carbohydrates, but many strains oxidize glucose.²³

Epidemiology

P. aeruginosa normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They often colonize hospital food, sinks, taps, mops, and respiratory equipment. Spread is from patient to patient via contact with fomites or by ingestion of contaminated food and water.

Infections

Pseudomonas aeruginosa causes infections in healthy individuals and those who are hospitalized or have a compromised immune system as a result of other diseases. A variety of human infections are commonly associated with this bacterium:

- Urinary tract infections
- Ventilator-associated pneumonia
- Surgical site infection
- Respiratory infections
- Ocular infections
- Ear infections (external otitis, malignant external otitis)
- Skin and soft tissue infections, including hot tub folliculitis, and osteomyelitis
- Burn sepsis

Individuals with compromising conditions, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes have an increased risk of acquiring an infection and developing complications.

Antimicrobial Susceptibility

Pseudomonas aeruginosa is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed, making susceptibility testing essential.²⁴

Antibiotic resistance

The discovery of antibiotics in the mid-twentieth century revolutionized the management and treatment of infectious disease caused by bacteria. Infections that would normally have been fatal were now curable. Since then, antimicrobial agents (antibiotics and related medicinal drugs acting on bacteria, viruses, fungi and parasites) have saved the lives and eased the suffering of millions of people. Today, antibiotics are crucial not only for the treatment of bacterial infections, but also for prophylactic coverage of high risk patients e.g. those in intensive care, organ transplants, cancer chemotherapy and prenatal care. However, these gains are now seriously jeopardised by the rapid emergence and spread of microbes that are resistant to antimicrobials.

The mass production of penicillin in 1943

dramatically reduced illness and death from infectious diseases caused by bacteria. However, within four years, bacteria began appearing that could resist the action of penicillin. Pharmaceutical companies fought back by developing other types of antibiotics. After more than 50 years of widespread use of these “miracle drugs”, antibiotics are no longer as effective as they once were. Virtually all important bacterial infections in throughout the world are becoming resistant. And even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.²⁵

From these microbes resistant to antibiotics:

Methicillin-resistant *Staphylococcus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections. MRSA infections are very difficult to cure because MRSA strains are resistance against almost all clinically available antibiotics (Adwan and Mhanna, 2008). MRSA infections that are acquired by persons who have not been recently hospitalized or had a medical procedure (such as dialysis, surgery and catheters) are known as Healthcare associated MRSA (HA MRSA) first appeared in the 1960s and has typically been linked to persons with health care associated risk factors such as hospitalization or nursing home care, chronic dialysis, antibiotic treatment, or exposure to invasive devices or procedures. HA MRSA is a highly resistant and important nosocomial pathogen in both acute care and long term care settings and causes infections associated with increased morbidity, mortality, and cost when compared to infections due to susceptible strains of *S. aureus* (Cuaresma *et al.*, 2008). Beginning in the 1990s community associated MRSA (CA MRSA) infections emerged in persons having none of the risk factors associated with MRSA in the past. CA MRSA is currently defined as an infection with MRSA in a person who does not have any prior history of a health care exposure such as hospitalization, surgery, permanent intravenous lines or other indwelling devices, or hemodialysis.²⁶ CA-MRSA infections are usually manifested as skin infections, such as pimples and boils, and occur in otherwise healthy people. They are often misdiagnosed as “spider bites” and can cause serious infections if not treated early (www.bop.gov).

Multidrug-resistant *Pseudomonas aeruginosa*

Pseudomonas aeruginosa also causes nosocomial

infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. A main problem is the emergence of multidrug-resistant *P. aeruginosa* strains resistant to different antimicrobial agent classes. Perhaps, this high degree of multidrug resistance related to the presence of antibiotic efflux systems which provide resistance to multiple antimicrobial agents.²⁷

Multidrug-resistant Enterobacteriaceae

Multidrug-resistant Enterobacteriaceae, mostly *Escherichia coli*, produces extended-spectrum β lactamases (ESBLs) such as the CTX-M enzymes. These enzymes were named for their greater activity against cefotaxime than other oxyimino-beta-lactam substrates such as ceftazidime, ceftriaxone, or cefepime have emerged within the community setting as an important cause of urinary tract infections (UTIs). Recent reports have also described ESBL-producing *E. coli* as a cause of bloodstream infections associated with these community-onsets of UTI.²⁸

INTRODUCTION OF ANTI-INFLAMMATORY

Plants are one of the important sources of medicine. The application of plants as medicine dates back to prehistoric period. Several indigenous drugs used in modern medicine have figured in ancient manuscripts such as Rigved, the Bible, and Quran. Over six thousand years ago, the ancient Chinese were the first to use the natural vegetation as medicine. In India, the Ayurvedic system of medicine has been in use for over three thousand years. Hippocrates, the 'Father of Medicine' was the first to give a scientific explanation of diseases. Indian system's of medicine includes Ayurveda, Siddha, Unani, Tibetan and Naturopathy. Herbal therapy provides rational means for the treatment of many internal diseases which are considered to be obstinate and incurable in other systems of medicine. It aims at both the prevention and cure of diseases.

HISTORY OF HERBAL DRUGS

Thousand years ago many natural drugs were identified for combating human ailments either by instinct or intuition or trial and error methods. The earliest mention of medicinal use of plants has been used found in '*Rig Veda*', which was written between 4000 and 1600 B.C. In the '*Atharva Veda*', we find the more varied use of drugs. It is the '*Ayurveda*' which is considered as an '*Upa Veda*' that definite properties of drugs and their uses have been given in great detail. '*Charka samhita*' is another earliest treatise on '*Ayurveda*' (600 BC), which lists a total of 341 plant produce for use health management. '*Sushruta Samhita*' also dealt with plants related to medicine.

Dhanvantari and *Nagarjuna* were the well known person with an intimate knowledge of the characteristics of medicinal plants. *Rauwolfia*, which has acquired world wide popularity, finds mention in ancient Hindu scriptures as well as in the monumental work of *charaka*. The plant was mentioned as useful antidote for snake bites and insect stings.²⁹

Their effectiveness, easy availability, low cost, and comparatively being devoid of serious toxic effect (time tested) popularized herbal remedies. Medicinal plants have their values due to the presence of chemical constituents, commonly known as secondary metabolites,

present in various plant tissues. These substances are alkaloids, glycosides, essential and fatty oils, resins, gums, mucilage, tannins etc. of large use. These active principles may be present in the organs of the plant, viz-roots, seeds, leaves, bark, wood etc...³⁰

Popularity of herbal medicine over allopathic medicine is because of the following reasons:-

- Low cost of medicine
- Relatively free from side effects
- Cures the root cause and remove it
- Cures for many obstinate diseases
- Easy availability³¹

SCOPE OF HERBAL MEDICINES:

Several civilizations across the globe have herbs in different forms. Before the advent of modern medicine herbs were used in the form of powders, pastes, tablets, galenicals like tinctures, infusion, decoction, oils saturated with herbs, oils, poultice etc. Herbs have been used in India in Ayurveda, Sidda, Unani systems of medicine, collectively known as indigenous system of medicine. The modernized method of formulating herbal medicines has yielded a new system of herbal medicine, popularly known as phytomedicine. The Ayurvedic medicines can be classified as like Charak samhita, Sushrut samhita, Bharat Bheshaj Ratnakar etc. and Ayurvedic proprietary medicine.

In developed countries, particularly in the United States of America, the herbal formulations are included in the list of food supplements and are considered as alternative or complementary system of medicines. Several clinical trials on herbal extract have been undertaken to prove their safety and efficacy using protocols acceptable to the modern medicines. Many developed countries have realized the usefulness of herbs as a source of starting material (lead compounds) in the process

of drug discovery. The chances of getting new drugs from herbs are more than getting new drugs from synthetic source because of availability of literature on plant activity. Many biomarkers have been isolated and identified.

There is fundamental difference between the indigenous system of medicine and modern medicine. The modern medicine makes use of single ingredient whereas the indigenous system of medicine believes that different constituents of herbs have synergistic effect. It has been shown that mode of action differs when effect of isolation biomarkers is compared with the total extracts.³²

2. PAIN

Algesia (pain) is an ill-defined, unpleasant sensory and emotional experience associated with actual or potential tissue damage, which varies from person to person and in the same person, from time to time. Unrelieved acute pain can cause chronic pain and long standing pain can cause anatomical and even genetic changes in the nervous system. Pain is warning signal, primarily protective in nature, but causes discomfort and suffering. Excessive pain may produce other effects such as sinking sensation, apprehension, sweating, nausea, palpitation, and rise or fall in BP, tachypnoea

TYPES OF PAIN

- Stimulation of a nociceptor, due to a chemical, thermal, or mechanical event that has the potential to damage body tissue, may cause nociceptive pain³³
 - Damage to the nervous system itself, due to disease or trauma, may cause neuropathic (or neurogenic) pain. Neuropathic pain may refer to peripheral neuropathic pain, which is caused by damage to nerves, or to central neuropathic pain, which is caused by damage to the brain, brainstem, or spinal cord.
 - Nociceptive pain and neuropathic pain are the two main kinds of pain when the primary mechanism of production is considered. Nociceptive pain may be classified further in three types that have distinct organic origins and qualities.
1. **Superficial somatic pain (or cutaneous pain):** Is caused by injury to the skin or superficial tissues.

DIAGNOSIS OF PAIN

The medical community is full of tests to try to find the cause of pain or disease. However, the real cause of your chronic, severe pain may be difficult to diagnose using labs and diagnostic tests. Imaging tests are also called radiological test. With these tests, the doctor uses different technologies to get a better picture of what's going on in

Cutaneous nociceptors terminate just below the skin, and due to the high concentration of nerve endings, produce a sharp, well-defined, localized pain of short duration. Examples of injuries that produce cutaneous pain include minor wounds, and minor (first degree) burns.

2. **Deep somatic pain:** It originates from ligaments, tendons, bones, blood vessels, fasciae, and muscles. It is detected with somatic nociceptors. The scarcity of pain receptors in these areas produces a dull, aching, poorly-localized pain of longer duration than cutaneous pain: examples include sprains, broken bones, and myofascial pain.
3. **Visceral pain:** Originates from body's viscera, or organs. Visceral nociceptors are located within body organs and internal cavities. The even greater scarcity of nociceptors in these areas produces pain that is usually more aching or cramping and of a longer duration than somatic pain. Visceral pain may be well localized, but often it is extremely difficult to localize, and several injuries to visceral tissues exhibit "referred" pain, where the sensation is localized to an area completely unrelated to the site of injury.³⁴

CAUSES OF PAIN

Pain can be caused by many different factors. Conditions that accompany normal aging may affect bones and joints in way that cause pain. Normal body pain is formed due to some common health conditions such as,

- Muscle Knots
- Sensitization
- Brain pain
- Pathological sensitization
- Vitamin D deficiency
- Muscle tension and contracture
- Referred pain
- The pain of stuckness
- Drug side effect, especially and statins
- bisphosphonates
- Analgesic rebound.³⁵

the body-with the bones, soft tissues, and organs.

Here are the most common imaging tests:

- X-ray
- CT scan
- MRI
- Nerve blocks

- *Discography*
- Myelogram
- *Ultrasound imaging*³⁶

TREATMENT OF PAIN

Pain is complex, so there are many treatment options - medication, therapies, and mind – body techniques. Some measures you can take to relieves pain from injuries

- Resting the area of the body where you are experiencing aches and pains.
- Applying ice to the affected area to help relieves pain and reduce inflammation.
- Taking an over-the- counter pain reliever, or **analgesic drugs** such as Acetaminophen, NSAIDs such as aspirin, ibuprofen etc.³⁷

ANALGESICS

A drug that selectively relieves pain by acting in the CNS or on peripheral pain mechanisms, without significantly altering consciousness. A wide range of drugs are used to control pain. They range from mild over-the-counter (OTC) drugs, such as aspirin and acetaminophen, to strong general anaesthetics. Drugs that relieve pain often reduce fever and inflammation that are used to treat conditions such as

- ❖ Mild to moderate pain caused by injury or surgery
- ❖ Fever, headaches, and painful menstruation
- ❖ Rheumatoid arthritis (a chronic inflammatory disease of the peripheral joints)
- ❖ Osteoarthritis (a chronic disease that involves wear and deterioration of joints in the body, causing inflammation)
- ❖ Chronic pain associated with cancer, AIDS, multiple sclerosis, or sickle cell disease

Some commonly used drugs are

- *Acetaminophen*
- Acetanilide
- *Morphine*
- Aspirin
- *Caffeine*

6. MATERIALS AND METHODS:

Plant collection and identification

Pinus longifolia is an aromatic much branched erect herb with 4 angled stems, bearded nodes leaves. It is a common weed of open lands. For the present study fresh plants were collected from locality and brought to laboratory in air tight polythene bags for further processing.

Preparation of leaf extract

For the preparation of leaf extract, fresh leaves were collected in a beaker and washed several times with water to remove the dust and finally with double distilled water. 10 g washed leaves were cut into fine pieces and crushed with the help of mortar and pestle in 100 ml double distilled water. After grinding the aqueous extract was taken in 250 ml beaker and boiled for 10 min at 80 °C temperature. The plant extract was allowed to cool at room temperature and then filtered with whatman filter paper. The filtrate was centrifuged for 20–25 min at 10000 rpm, the supernatant was collected and stored at 4 °C. This filtrate was used as a stabilizing and reducing agents.

Phytochemical analysis

The Methanolic extract of *Pinus longifolia* were subjected to qualitative phytochemical tests for different constituents such as alkaloids, carbohydrates, glycosides, flavonoids, phenolic compounds, proteins, and free aminoacids and triterpenoids.

1. Test for carbohydrate

Small quantity of extract was dissolved in 5ml of water and filtered.

Molisch test

The filtrate was treated with a few drops of α -naphthol (20% in ethyl alcohol). Then 1 ml of concentrated H_2SO_4 was added along the sides of inclined test tube and observed for formation of violet coloured ring at the interface.

1. Test for glycosides and anthroquinones Borntrager's test

A small amount of Methanolic extract was hydrolysed with hydrochloric acid for few hours on water bath and the hydrosylate was extracted with benzene. The benzene layer was treated with dilute ammonia solution and observed for the formation of reddish pink colour.

Legal test

The extract was dissolved in pyridine and made alkaline with few drops of 10% NaOH and freshly prepared sodium nitroprusside was added and observed for formation of blue colour.

1. Test for flavonoids Ammonia test

Filter paper strips were dipped in the dilute solution of the extract, ammoniated and observed for colour change from white to yellow.

1. Test for Tannins and Phenolic compounds

The extract was dissolved in distilled water and dissolved into three portions. Sodium chloride (10%) was added to one portion, 1% gelatine to second portion and gelatine salt reagent to third portion. Precipitation with later or both gelatin salt reagents was indicative of the presence of tannins. Precipitation with salt solution indicates a false positive test. Positive tests were further confirmed by the addition of a few drops of dilute ferric chloride (1%FeCl₃) to the test extract which gave blue or green black coloration.

Test for Proteins and Aminoacids

Small amount of extract was dissolved in distilled water and filtered.

Biuret's test

To the ammoniated alkaline filtrate add 2-3 drops of 0.002% copper sulphate and observed for appearance of red or violet colour.

Millon's test

To 2 ml of filtrate 5-6 drops of millons reagent (1 g of mercury + 9 ml of fuming nitric acid solution) was added and observed for red precipitates.

Ninhydrin test

To the filtrate lead acetate solution was added to precipitate tannins and filtered. The filtrate was spotted on paper chromatogram and sprayed with ninhydrin reagent and heated at 110°C for five minutes and observed for red or violet colour.

Xanthoprotein test

To the filtrate a few drops concentrated nitric acid was added by the side of test tube and observed for appearance of yellow colour.

Test for sterols and triterpenes

The extract was refluxed with alcoholic potassium hydroxide until the completion of saponification. Then the mixture was diluted with distilled water and extracted with diethyl ether. The ethereal extract was evaporated and the unsaponifiable matter was subjected to the following tests.

Libermann- Buchard's test

The ether soluble residue was dissolved in chloroform and a few drops of acetic anhydride was added followed by a few drops concentrated sulphuric acid from sides of the test tube and observed for the formation of blue to blue- red colour.

Salkowski's reaction

To the ether soluble residue 2 ml of concentrated sulphuric acid was added and observed for the formation of yellow ring at the junction which turns red after one minute.

ANTI- MICROBIAL ACTIVITY STUDIES ON EXTRACT AND FRATIONS OF PINUS LONGIFOLIA

Physical nature and percentage yield of extracts

The percentage yield and physical status of the methanolic leaf extract (MEPL) and chloroform (CSC) and aqueous fractions *Pinus longifolia* are showed in the TABLE Yield of the extracts calculated with reference of the weight raw material used. For fractions, the yield was calculated with respect to corresponding methanolic extract.

Table-6.2: Percentage yield and physical status of the methanolic extract and fractions of *Tulasi*

Extract/Fraction	Code	Yield (%)
Methanolic	MEBM	12.5
Ethyl acetate	BME	6.5
Chloroform	BMC	5.3
n-hexane	BMH	6.1
Aqueous	BMQ	3.3

Antibacterial action:

Antibacterial activity

Generally the antibacterial activity of a compound is expressed in terms of its ability to inhibit the growth of bacteria in nutrient broth or agar media. The bacterial growth inhibition can be measured by two methods.

a). Cup plate method

b). Serial dilution method

Bacterial Culture medium:

Nutrient broth has been used for the preparation of inoculum of the bacteria and nutrient agar is used for the evaluation of antibacterial activity.

Antibacterial activity

Antibacterial activity by Cup plate Agar Diffusion method by measuring the zone of inhibition in mm.

Materials:

- Nutrient Broth Media
- Nutrient Agar media
- Streptomycin (standard)
- Dimethyl Sulfoxide (DMSO)
- Distilled Water

Required Equipment:

- Laminar air flow cabin

Table 6.3: Composition of the nutrient agar medium:

Peptone	5gm
Sodium chloride	5gm
Beefextact	1.5gm
Yeast extract	1.5gm
Agar	1.5gm
Distilled water up to	1000mL
pH	7.4±0.2

Procedure:

The test organisms were sub cultured using nutrient broth medium. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37±1°C for 24 hours they were stored in refrigerator. The stock cultures were maintained. Bacterial inoculum was prepared by transferring a loopful of culture to nutrient broth in conical flask. The flasks were incubated at 37±1°C for 48 hours before the experimentation.

Solution of test compound was prepared by dissolving the sample in DMSO. A reference standard for both Gram positive and Gram negative bacteria was made by dissolving accurately weighed quantity of Moxifloxacin and Streptomycin in sterile distilled water.

The nutrient agar medium was sterilized by autoclaving at 121°C for 15min. The petriplate, tubes and flasks plugged with cotton were sterilized in hot air oven at 160°C for an two hours. Into each sterilized petriplate, about 25mL of molten nutrient agar medium inoculated with the respective strains of bacteria was transferred aseptically. The plates were left at room temperature to allow solidification. In each plate, cups of 5 mm diameter were made with sterile borer. Then 100µl of the test solution was added to the respective cups aseptically and labeled accordingly. The plates were kept undisturbed for at least 2 hours in refrigerator to allow diffusion of the solution properly into the nutrient agar medium. After

- Incubator
- Refrigerator
- Micropipettes
- Culture plates
- Boiling tube
- Eppndrof tubes

Evaluation for Antimicrobial Activity:

The antibacterial activity of substituted chalcones (**3a-3e**) and their derivatives 4,6 diaryl oxazines (**4a-4d**) had been assayed against four different strains of bacteria by cup-plate agar diffusion method by measuring the zone of inhibition.

the incubation of the plates at 37±1°C for 24 hours, the diameter of the zone of inhibition surrounding each of the cups was measured with the help of the scale and tabulated.

Standard drugs used : Streptomycin, Moxifloxacin

ANTI-INFLAMMATORY ACTIVITY**Animals**

Swiss albino mice weighing 20-25 gm wistar rats weighing 150- 200 gm were used for this study. The animals were obtained from animal house. On arrival, the animals were placed randomly and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. A12: 12 light: day cycle was followed. All animals were allowed to free access to water and bed with standard commercial pelleted chow. All the experimental procedures are protocols used in this study were reviewed by Institutional Animal Ethics Committee.

Acute Toxicity Studies**The acute toxicity was performed according to OECD guidelines**

423. The selected albino rats were used for toxicity studies. The animals were divided into five groups of three in each. The animals were fasted overnight prior to the acute experimental procedure. Extract was given orally to rats at the graded doses like 10, 30, 100, & 300 mg/ kg body weight. Immediately, after dosing, the animals were observed continuously for first

four hours for behavioural changes were closely observed for hyperactivity, ataxia, convulsion, salivation, tremors, diarrhoea, lethargy, sleep. They were then kept under observation up to 14 days after drug administration to determine the mortality, if any. One-tenth and one-fifth of the maximum tolerated dose (200 and 400 mg/ kg, body weight) of Methanol leaf extract of *Nymphaea stellata*. Selected to evaluate

analgesic, anti-inflammatory and antipyretic activity studies in rats.

Statistical analysis

The values of statistical analysis were expressed as mean values $\pm$ SEM. The results of the experiment were performed as changes of percentage from control value. Data was analyzed by ANOVA followed by Dunnett's multiple comparison tests. $p \leq 0.05$ was considered to be statistical significance.

RESULTS:

8. Qualitative phytochemical Evaluation of *Pinus longifolia*.

Parameters	value
1. Alkaloid	+
2. Carbohydrates	+
3. Glycosides	-
4. Flavonoids	++
5. Tannins & Phenolic compounds	+
6. Proteins & Amino acids	+
7. Saponins	+
8. Sterols or Triterpenes	+

++: high content, +: moderate, -: Negative,

From the qualitative phytochemical analysis of ethanolic extract of *Pinus longifolia*. It content alkaloids, Tannins, phenolic compound, sterols, saponins, protein and amino acids and high amount of flavonoids.

RESULTS AND DISCUSSIONS ON ANTI-MICROBIALB ACTIVITY:

Table: Zone of inhibition of solvent extract of *Pinus longifolia* against *E.Coli*:

Compounds	50 μ g/ml (mm)	100 μ g/ml (mm)	1000 μ g/ml (mm)
MEBM	6	10	12
BME	10	12	16
BMC	11	15	18
BMH	8	10	14
BMQ	12	18	20
CPN	12	16	20
MXN	14	18	24

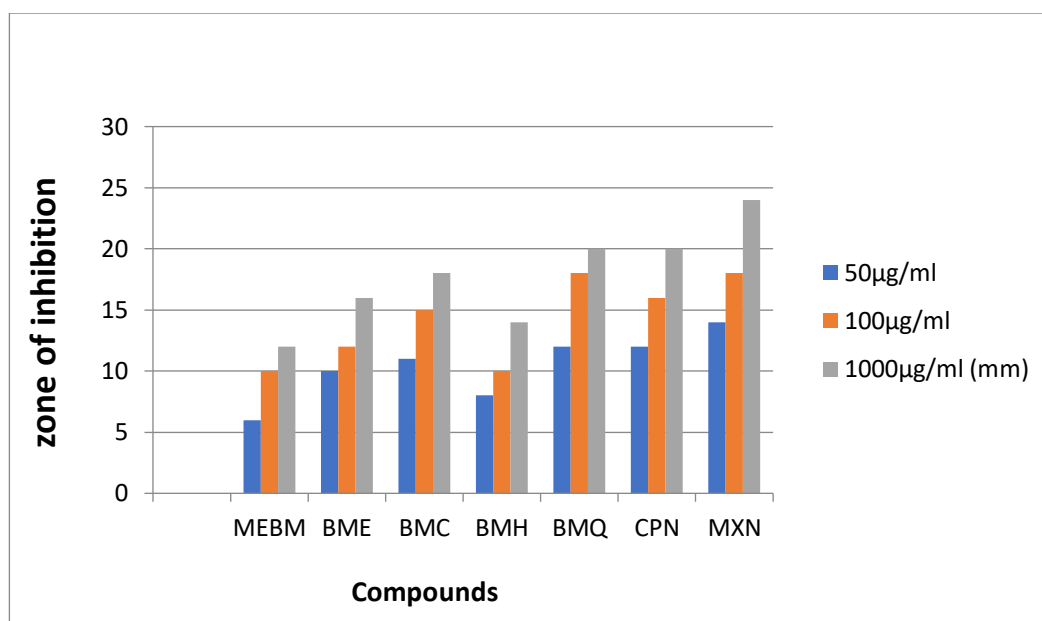
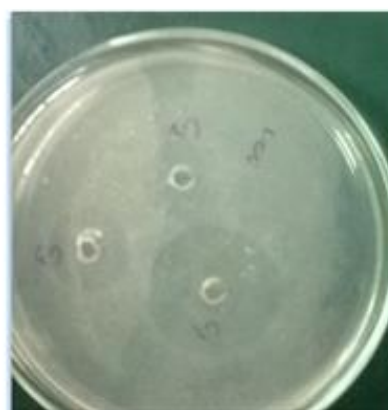


Figure: Zone of inhibition of solvent extract of *Pinus longifolia* against *E. coli*

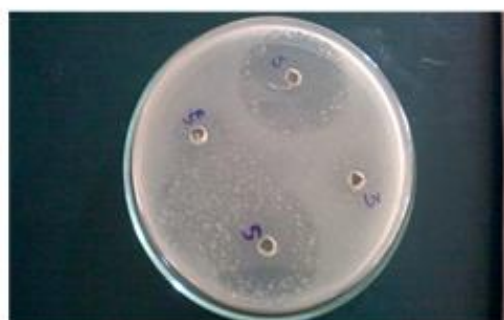
Antibacterial activity of compounds against *E. coli*



MEBM



BMC

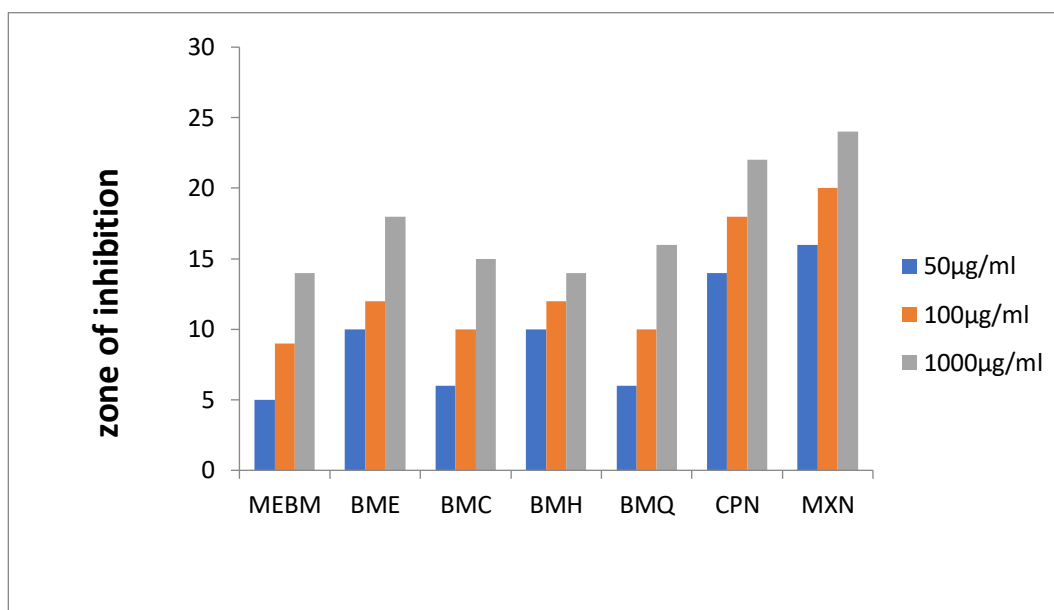
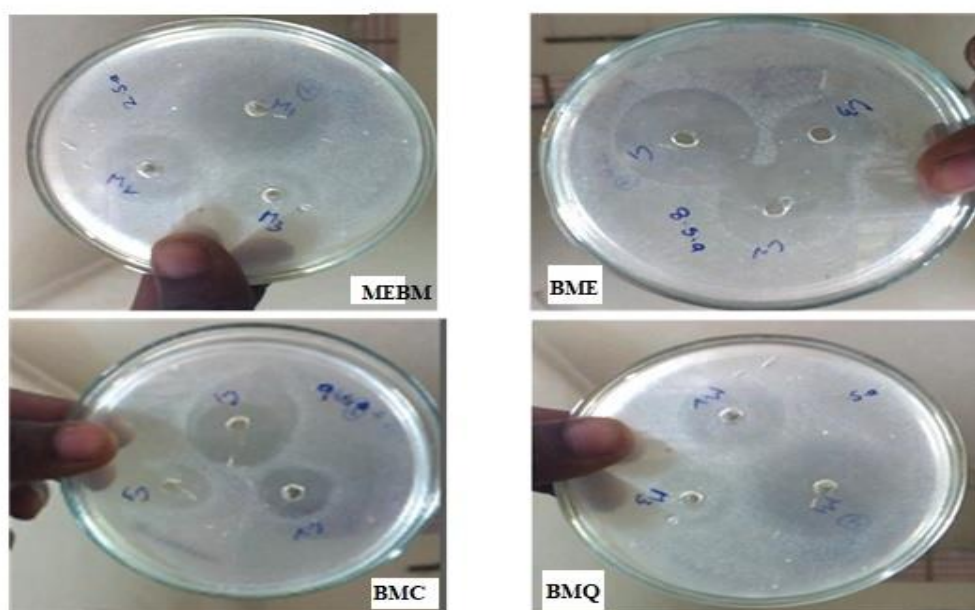


BME

Figure: Zone of inhibition of solvent extract of *Pinus longifolia* against *E. coli*

Table: Zone of inhibition of *Pinus longifolia* against on *Streptococcus aureus*:

Compounds	50µg/ml	100µg/ml	1000µg/ml
MEBM	5 mm	9 mm	14 mm
BME	10 mm	12 mm	18 mm
BMC	6 mm	10 mm	15 mm
BMH	10 mm	12 mm	14 mm
BMQ	6 mm	10 mm	16 mm
CPN	14 mm	18 mm	22 mm
MXN	16 mm	20 mm	24 mm

**Figure: Zone of inhibition of *Pinus longifolia* against on *Streptococcus aureus*:****Antibacterial activity against *staphylococcus aureus*****Figure: Zone of inhibition of *Pinus longifolia* against on *Streptococcus aureus*:**

Results and discussions of Antimicrobial Activity:

The Extracted compounds **MEBM**, **BME**, **BMC**, **BMH**, **BMQ** has been evaluated for their antimicrobial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria by measuring the zone of inhibition. The results have been compared with a broad spectrum antibacterial agent Ciprofloxacin as standard drug.

- All the Extracted compounds (BMC & BMQ), were found to be effective antibacterial agents.
- The compounds MEBM showed minimum activity against *E.coli* with zone of inhibition 6mm, 10mm, 12mm.
- The compounds BMC showed maximum activity against Gram positive bacteria *E. Coli* at 100µg/ml with zone of inhibition 15mm. Among all those compounds, MEBM, BMC and BMQ showed Poor activity against Gram-positive (*Staphylococcus aureus*) with zone of inhibition of 5mm, 6mm and 5mm.

DISCUSSION:

Evaluation of Anti-Bacterial effect Methanolic leaves extract of *Pinus longifolia*. Deals with the exploration of pharmacological and phytochemical screening of the selected Indian medicinal plant *Pinus longifolia*.

The method used for Methanolic extract was found to be simple and reproducible. The Methanolic extract of *Pinus longifolia* demonstrated significant Anti-Microbial properties in experimental animals in this study. These activities may be attributed to the various Phytoconstituents of Methanolic extract of *Pinus longifolia* such as flavonoids, tannins, saponins, alkaloids, anthocyanins, glycosides, polyphenols, and steroids. However, further experimental studies are required to explore the exact mechanism of actions and next level of clinical trials to generate novel drugs. This might prove helpful to use its immense therapeutic efficacy as a potent Anti-Microbial phytomedicine.

The *Pinus longifolia* has shown significant Anti-Bacterial effects. These effects maybe because of the presence of phytochemicals such as flavonoids, tannins and Terpenoids present in the plant extract.

The Present study showed that the Methanolic leaves extract of *Pinus longifolia*, posses peripheral and central Anti-Bacterial activity in

animal model. The *Pinus longifolia* leaves extract showed Anti-Bacterial activity in different animal model. Flavonoids and tannins are the major constituents of *Pinus longifolia* leaves, which may be responsible for its Anti-Bacterial activity.

Further detailed study on *Pinus longifolia plant* using different flogestic agents in this area will enable us to understand the mechanism of action underline the above mention activity.

REFERENCES:

1. B. Nitha, A.B. Remashree and Indira Balachandran. Antibacterial Activity Of Some Selected Indian Medicinal Plants. IJPSR, 2012; Vol. 3(7): 2038-2042.
2. Chaudhary G, Goyal S, Poonia P. Lawsonia inermis Linnaeus: A Phytopharmacological Review. International Journal of Pharmaceutical Sciences and Drug Research 2010; 2(2): 91-98.
3. Goyal BR, Goyal RK, Mehta AA. Phyto-Pharmacognosy of *Archyranthes aspera*: A Review. Pharmacognosy Reviews 2008; 1:1.
4. Cragg GM, Newman DJ, Sander KM. Natural products in drug discovery and development. Journal of Natual Products 1997; 60:52-60.
5. Dean DA, Burchard KW. Fungal infection in surgical patients. American Journal of Surgery 1996; 171: 374-382.
6. Gonzalez CE, Venzon D, Lee S et al. Risk factors for fungemia in children infected with human immunodeficiency virus: a case control study. Clinical Infectious Diseases 1996; 23: 515-521.
7. VaghasiyaY, Chanda SV Screening of Methanol and Acetone Extracts of Fourteen Indian Medicinal Plants for Antimicrobial Activity. Turkish Journal of Biology 2007; 31:243-248.
8. Soulsby EJ. Resistance to antimicrobials in humans and animals. Britsh Journal of Medicine 2005; 331: 1219-1220.
9. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews 1999; 12: 564-82.
10. Udayan PS, Balachandran I: Medicinal Plants of Arya Vaidya Sala Herb Garden. Department of Publication Arya Vaidya Sala, Firt Edition 2009.
11. Tirtha SS, "The Ayurveda Encyclopedia- Natural Secrets to Healing, Prevention and Longevity", 2007.
12. S. Chaudhari, N. Shaikh, "Gaduchi-the best ayurvedic herb", The Pharma Innovation Journal, 2(4), 97-102, 2013. International Journal of Scientific Research and Review

- Volume 7, Issue 12, 2018 ISSN NO: 2279-543X Page No: 593.
13. U. Spandana, S.L. Ali, T. Nirmala, M. Santhi, S.D. SipaiBabu, "A Review on *Tinosporacordifolia*", International Journal of Current Pharmaceutical Review and Research, 4(2), 61-68, 2013.
 14. KN. Aiyer, M. Kolammal, Pharmacognosy of Ayurvedic Drugs, Series 1. 1st ed Trivendram, The central Research institute, 1963.
 15. KR. Kirtikar and BD. Basu, Indian Medicinal Plants. Second Edition, International Book Distributors, Dehradun, 2005.
 16. Database on Medicinal Plants Used in Ayurveda, Vol. 3, CCRAS, Reprint, New Delhi, 2005.
 17. KM. Nadkarni KM, AK. Nadkarni, Indian Materia Medica. Popular Prakashan Private Limited Mumbai, 3, 1976.
 18. Kirtikar KR, Basu BD Indian medicinal plant (2nd ed). Bishen Singh Mahendra Pal Singh, Dehra Dun, vol I, 499–502, 1975.
 19. Perez C, Paul M, Bazerque P. An Antibiotic assay by the agar well diffusion method. *ActaBio Medica Exp* 1990; 15: 113-115.
 20. Westh H, Zinn CS, Rosdahl VT, Sarisa Study Group. An international multicenter study of antimicrobial consumption and resistance in *S. aureus* isolates from 15 hospitals in 14 countries. *Microbial Drug Resistance*. 2004. 10:169 -176.
 21. Shariff ZU. Modern Herbal Therapy for Common Ailments. Nature Pharmacy Series. Spectrum Books Ltd., Ibadan, Nigeria in Association with Safari Books (Export) Ltd. UK, Vol.1,2001: 9-84.
 22. Parekh J and Chanda S Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *African Journal of Biomedical Research* 2007; 10: 175 – 181.
 23. Srinivasan D, Nathan S, Suresh T, Perumalaswamy O. Antimicrobial activity of certain Indian Medicinal Plants used in folkloric medicine. *Journal of Ethnopharmacology* 2001; 74: 217-220.
 24. Mayers D.L., Lerner S.A., Ouelette M. vol. 2. Springer Dordrecht Heidelberg; London: 2009. (Antimicrobial Drug Resistance C: Clinical and Epidemiological Aspects). pp. 681–1347.
 25. Jorgensen J.H., Ferraro M.J. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin. Infect. Dis.* 2009;49:1749–1755.
 26. Caron F. Antimicrobial susceptibility testing: a four facets tool for the clinician. *J. Des. Anti-Infect.* 2012;14 186-174.
 27. Nijs A., Cartuyvels R., Mewis A. Comparison and evaluation of Osiris and Sirscan 2000 antimicrobial susceptibility systems in the clinical microbiology laboratory. *J. Clin. Microbiol.* 2003;41:3627–3630.
 28. Denes É., Hidri N. Synergie et Antagonisme en Antibiothérapie. *Antibiotiques*. 2009;11:106–115.
 29. Sharma S. et al "Topical Drug Delivery System" available from <http://www.pharmainfo.net/> accessed on 12/07/2015.
 30. Laithy HM. and El shaboury KMF. The development of Cutina Lipogels and gel microemulsion for topical administration of fluconazole. *Ame Pharm Sci. PharmSciTech*. 2003; 3:10 25.
 31. Kshirsagar N A. Drug Delivery Systems. *Ind. J. Pharmacol.* 2000; 32:S54- S61.
 32. Dadwal Meenakshi, emulgel: a novel approach to topical drug delivery International journal of pharma and bio sciences, 4 (2013), 847-856.
 33. Rashmi M. Topical gel: A review august vol. 2008; available from <http://www.pharmainfo.com>
 34. Sharma S. Topical preparations are used for the localized effects at the site of their application by virtue of drug penetration into the underlying layers of skin or mucous membranes. *Pharmaceutical reviews* 2008; 6:1.
 35. Stanos SP. Topical Agents for the Management of Musculoskeletal Pain .*J Pain Symptom Manage* March 2007; 33.
 36. Jain A, Deveda P, Vyas N, Chauhan J et al. Development Of Antifungal Emulsion Based Gel For Topical Fungal Infection(S). *IJPRD* 2011; 2(12).
 37. Bruton L, Keith P, Blumenthal D, Buxton Z. Goodman & Gillman's Manual of Pharmacology & Therapeutics. Mc Graw's Hill. 2008. pp.1086-1094.
 38. Cecv G. Preclinical characterisation of NSAIDs in ultradeformable carriers or conventional topical gels. *International journal of pharmaceutics*; 2008.
 39. Bhoyar N, Giri T.K, Tripathi D.K, Alexander .A and Ajazuddin: Recent advances in novel drug delivery system through gels: review, *J. Pharm. allied health sci*, 2012, 2(2), 21-39.
 40. Khullar Rachit, Saini S, Seth N and Rana A C: Emulgels: A surrogate approach for topically used hydrophobic drugs, *IJPBS*, 2011, 1 (3), 117-128.

41. Prajapati Mehulkumar N, Patel M R, Patel K R & Patel N M: Emulgels: a novel approach to topical drug delivery, IJUPBS, 2013, 2(1), 134- 148.
42. Joshi baibhav, Singh Gurpreet, Rana A C,

Saini Seema & Singla Vikas: Emulgel: A comprehensive review on recent advancement on topical drug delivery, IRJP, 2011, 2(11).