A study on phytochemical profiling and antimicrobial activity of *Cymbopogon citratus* (Lemon grass)

THESIS

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By

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CERTIFICATE

This is to certify that the thesis entitled ***"A study on phytochemical profiling and antimicrobial activity of Cymbopogon citratus (Lemon grass)"*** submitted in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **Biotechnology** of the College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut is a record of bonafide research work carried out by **Ms. Saumya Jaiswal, ID. No. 4890,** under my supervision, and no part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the work of research have duly acknowledged.

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CERTIFICATE

We, the undersigned, members of the Advisory Committee of **Ms. Saumya Jaiswal, ID. No. 4890**, a candidate for the degree Master of Technology I Biotechnology agree that the thesis entitled ***"A study on phytochemical profiling and antimicrobial activity of Cymbopogon citratus (Lemon grass)"*** may be submitted by **Ms. Saumya Jaiswal** in partial fulfillment of the requirements for the degree.

(Pankaj Chauhan)
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(Ravindra Kumar)	**(Neelesh Kapoor)**	**(Shewta Mishra)**	**(Rekha Dixit)**
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ABBREVIATION

MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NB	Nutrient Broth
PSI	Pound Per Square inch
SDA	Sabouraud Dextrose Agar
ZDI	Zone Diameter of Inhibition

CHAPTER 1 INTRODUCTION

Cymbopogon comes from the Greek words "kymbe - pogon," which means "boat-beard" (owing to the shape of the flower spikes) and "*citratus*," which means "lemon-scented leaf" (Bhardwaj, 2020). The Poaceae family includes the genus *Cymbopogon*, also known as lemon grass. It is native to southwest Asia (southern India and Sri Lanka), but it currently grows wild all over the world, particularly in tropical and subtropical climates (Machraouim *et al.,* 2018). There are about 500 genus and 8,000 plant species in there (Barbosa *et al.,* 2008). *Cymbopogon citratus* grows in thick clumps that can grow up to 3 meters tall and have short rhizomes. The entire plant has a lemony aroma and a harsh flavour. It has upright, glabrous plane leaves that are more than 1 m long, 5-15 mm broad, with a whiter upper face and closed edge at the base, rough edges, and 4-5 mm long membranaceous or arid ligules. The leaves have a dark green upper surface and a light green lower surface. Inflorescences are erect, usually in pairs with 30-60 cm long terminal spiciform racemes. Sessile tiny spikes with ciliated borders, canaliculated ventral side, 4.5-5.0 mm long and 0.8-1.0 mm broad (Negrelle *et al.,* 2007 and Shah *et al.,* 2012). Flowering, on the other hand, has never been observed under cultivation due to the short harvesting season (Tajidin *et al.,* 2012).

Ayurvedic therapy makes considerable use of freshly cut and partially dried leaves. Coughs, elephantiasis, flu, gingivitis, headache, fever, hypertension, leprosy, malaria, ophthalmic pneumonia, neurological, gastrointestinal, and vascular problems are all treated with the grass (Karkala *et al.,* 2014). Antibacterial (Danlami *et al.,* 2011), antifungal (Ngueface *et al.,* 2012), antioxidant (Hanisa *et al.,* 2011 and Garg *et al.,* 2012), and anti-inflammatory (Figueirinha *et al.,* 2010) capabilities are among the pharmacological qualities of *Cymbopogon citratus.*

The therapeutic potential of medicinal herbs may be linked to the existence of secondary metabolites, according to Christopher E Ekpenyong (2014). The principal bioactive compounds

generated from *Cymbopogon citratus* leaves, stem, and roots are thought to be responsible for the plant's biological effects. Lemon grass secondary metabolites such as citral (3, 7-dimethyl-2, 6-octadienal), myrcene and citronellal have all been identified as anti-malarial chemicals. These isolated compounds have significant anti-plasmodium activity (Kpoviessi *et al.,* 2014). Ketones, alcohols, phenols, terpenes, flavonoids, saponins, steroids, tannins, alkaloids, geranial, terpernoids, polyphenols, esters, aldehydes, and fatty acids have all been isolated and studied as bioactive ingredients (Brugger *et al.,* 2019). Because of the concerns with antibiotic-resistant bacteria and antibiotic residues in animal products, as well as the threat to human health, there is a renewed and growing interest in finding alternatives to antibiotics for livestock treatment. Essential oils (plant extracts) have recently been employed as feed additives to increase cattle performance, particularly under intensive management systems (William *et al.,* 2001). *Cymbopogon citrate* is one of the plants that could have a substantial medicinal effect.

Phytochemicals are non nutritive bioactive plant chemicals that serve as antioxidants, enzyme stimulants, anti-bacterial agents, anti-cancer agents, and have hormonal effect (Akinmoladun *et al.*, 2007). The majority of plants that have high levels of these phytochemicals are referred to as medicinal plants. Some chemical compounds found in medicinal plants have been shown to have clear physiological effects on the human body (Yadav *et al.,* 2011). Traditional remedies and plant pharmaceuticals have been used to treat a variety of ailments for several decades since they are safer and have few or no adverse effects (Rabia, 2005, Parvath *et al.,* 2003 and Gore *et al.*, 2010).

According to the World Health Organization, almost 80% of the world's population relies on traditional medicines to meet their primary health care needs. Helminthes have long been a source of concern in medicine, and they continue to pose significant difficulties for both humans and animals. Despite numerous advances in understanding the mode of transmission and treatment of these

helminthes over the past few decades, there are still no effective products to control certain helminthes, and the indiscriminate use of some drugs has resulted in a number of cases of resistance. Since time immemorial, India's traditional system and folklore have claimed that medicinal plants, in whole or in part, have successfully treated a wide range of ailments, including antibacterial, anti-helmintic, and anti-inflammatory conditions. Citral, myrcene, citral, limonene, dipentene, heptenone, borncol, geranial, geraniol, myrcene, citronellol, 6-methyl-5-hepten-2 ona, and undecan-2-one are some of the bioactive compounds (Onawunmi *et al*., 1984). Lemon grass is a member of the Andropogan genus *Cymbopogam*, which belongs to the Germineae family. A vast genus in the family with around 500 identified species, eight of which are found in Iraq (Anonymous, 2005).The leaf blade is linear, tapering at both ends, and can reach 50 cm in length and 1.5 cm in width18. There are many species in this genus, but only two are economically important as cultivated plants: *Cymbopogon citratus* and *Cymbopogon flexuosus* (Sugumaran *et al.,* 2005 and Avoseh *et al.,* 2015). East Indian lemongrass, *Cymbopogon flexuosus*, is cultivated in Asia, whereas West Indian lemon grass, *Cymbopogon citratus*, is drought and low temperature resistant and thus can be cultivated across larger areas, making it more commercially important (Prins *et al.,* 2013). It has been grown for its medicinal benefits, as well as for garden decoration and insect repellent properties. It is a tropical plant that is widely planted as an ornamental plant in temperate climates. Plants are a valuable source of medicinal agents because they contain a variety of active constituents with high therapeutic value. Plants and herbs have held a special place in all cultures around the world from prehistoric times (Umar *et al.,* 2016). Plant based medications are widely utilised for the treatment of a variety of disorders since they are readily available and have a lower harmful effect on the recipient than manufactured drugs (Joshi *et al.,* 2012).

Herbal medications are becoming increasingly popular, and they now account for a significant

portion of the global drug market (Sandhya *et al.*, 2014). More than 75% of the world's population relies on medicinal plants to meet their fundamental health requirements. Because it has no side effects, plant based medicine has become a popular alternative to synthetic medicine (Ranade *et al*., 2015). The leaves of the *Cymbopogon citratus* plant are utilised in food, cosmetics, and pharmaceuticals. Lemon grass is an important medicinal plant with a wide range of uses in traditional medicine. It can also be used to treat HIV-related problems, particularly secondary bacterial infections (Umar *et al.,* 2016). Due to the presence of different secondary metabolites in lemon grass, it has long been used to treat a variety of medical ailments. It has been also used to treat fever, cough, elephantiasis, flu, leprosy, malaria and other digestive problems. Antimicrobial activity of lemon grass against various bacteria, fungi, protozoa has also been reported. There is a scarcity of scientific research and knowledge on the medicinal properties of lemon grass. Lemon grass's use in clinical settings has been limited due to a lack of scientific understanding (Ranade *et al*., 2015). The oil has been discovered to have bactericidal, antibacterial, and antifungal effects that are comparable to those of Penicillin (Lutterodt *et al.,* 1999). A male sex hormone agent is also included in the oil (Gupta *et al.,* 1993). It has antipyretic and analgesic effects. Lemon grass juice includes an inhibitor of carcinogenesis generated by cotton oil during the promotion stage. It's an anti tumor drug that's taken orally and combined with cyclodextrin to help patients live longer (Oshiba *et al.,* 1991 and Parekh *et al.,* 2007). Oil based preparations for removing gallstones have been developed (Elastal *et al.,* 2005). Due to its commercially important essential oils, *Cymbopogon citratus* is of tremendous interest and is frequently employed in food technology as well as traditional medicine. Due to the introduction of new diseases, people are becoming more aware of health issues. Because of the negative side effects associated with synthetic medications, treatment with plant-based medicine looks to be an alternative (Mirghani *et al.,* 2012).

Following objectives were undertaken in present study entitled "A study on phytochemical profiling and antimicrobial activity of *Cymbopogon citratus* (lemon grass)".

i. To extract Phytochemicals from *Cymbopogon citratus* leaf using different solvents.

ii. To check antibacterial & antifungal activity of *Cymbopogon citratus* leaf extract.

CHAPTER 2 REVIEW OF LITERATURE

Lemon grass, also known as citronella grass, is a plant that belongs to the Poaceae family and the genus *Cymbopogon*. *Cymbopogon* is a genus of about 140 species that can be found growing in semi-temperate and tropical parts of the Asian, American, and African continents. Only a few

species of lemon grass grow in Australia and Europe. Lemon grass, often known as 'Squinant' in English, is also known by a variety of different nicknames around the world. The aromatic grasses are members of the *Cymbopogon* genus, which generate volatile oils (Kumar *et al*., 2009 and Adhikari *et al*., 2013). The high citral content of this grass oil gives it a pronounced lemon scent, which is a distinguishing feature. The oil's redolence allows it to be used in soaps, detergents, and other products. It's used in the perfumery and culinary sectors because it's a good source of citral. It is also the raw ingredient for the production of ionones, which are the precursors to Vitamin A. (Viabhav *et al.,* 2013). Lemon grass includes a number of bioactive chemicals that make it therapeutic. There is a lot of evidence for its pharmacological applications (Kumar *et al.,* 2010). Herbal medicine is seen as a significant element of the healthcare business by more than two-thirds of the population in developing nations, according to the World Health Organization (WHO) (Okémy *et al.,* 2015). Apart from providing an overview of lemon grass, this review article focuses on its medical characteristics, which make it a useful herb for pharmaceutical and diagnostic purposes.

Taxonomic description

The genus *Cymbopogon* is named after the Greek words 'kymbe' (boat) and 'pogon' (beard). It has a large number of species, the most valuable of which being *Cymbopogon citratus* and *Cymbopogon flexuosus* as cultivated plants. However, *Cymbopogon citratus* is more resistant to drought and extreme temperatures, and as a result, it can be grown over a larger area, making it more commercially important.

Cymbopogon citratus leaves are often used as lemon flavouring in natural teas, prepared by decoction or infusion, or in finished natural products such as capsules, tablets, and lotions. Essential oils are well known for their usage in perfumery and as a key ingredient in Asian cuisine, but they're

also widely used in other industries (pharmaceutical and cosmetic) due to their bioactive molecules, which have a variety of healing properties. Genetic variety, habitat, and agronomic treatment of the culture, as well as a part of the plant, adulthood level, and extraction procedure, all influence the chemical makeup of *Cymbopogon citratus* crucial oil. Citral, a mixture of isomeric acyclic monoterpene aldehydes: geranial (trans-citral) and neral, is present in the essential oil of *Cymbopogon citratus* (cis-citral). *Cymbopogon citratus* has excellent antibacterial properties and can be used as an alternative treatment for enteric fever, infectious diseases of the respiratory system, and oral hygiene. It works by removing bacteria from the oral cavity and preventing periodontitis, plaque, and gingivitis. Furthermore, *Cymbopogon citratus* revealed high levels of overall phenolic and flavonoid content, as well as high free radical scavenging capacity and antioxidant activity. *Cymbopogon citratus* indicates correct anti-inflammatory, antidiabetic, hypolipidemic, Reno protective and cardioprotective, in addition to anticancer activities. Apart from this, *Cymbopogon citratus* possesses vasorelaxant, sedative and antitussive ability. In addition, the compound citral is employed in the fragrance industry as well as for wound cleansing and skin disease treatment in the form of gels or useful paper microencapsulated with essential oil for hand hygiene (Aimovi *et al.*, 2019). Lemon grass is a perennial monocotyledonous grass that can reach a height of 6 feet and a width of 4 feet. It blooms in clumps. . It has long, slender, drooping bright green leaves with a width of 1.3-2.5cm and a length of 3-feet. The leaves are simple and have full margins. On spikes, flowers grow. It has a long inflorescence that can reach 30-60cm in length. The term '*Cymbopogon*' comes from the flower arrangement of this aromatic grass. *Cymbopogon citratus* is a common Southeast Asian plant (Shah *et al.,* 2011). Lemon grass is a fragrant medicinal grass from the *Cymbopogon* genus. It is common in the semi-tropical and tropical regions of the Asian, American, and African continents. The considerable citral content material in this grass oil gives it a strong lemon aroma,

which is one of its key characteristics. The oil's redolence permits it to be used in soaps, detergents, and perfumes. It also demonstrates usefulness within the pharmaceutical sector. Lemon grass is now available in a wide variety of ethnopharmacological products. Apart from vitamins, it also contains a variety of bioactive chemicals such as alkaloids, terpenoids, flavonoids, phenols, saponins, and tannins, which are divided into alkaloids, terpenoids, flavonoids, phenols, saponins, and tannins. Lemon grass fitness-restorative properties can be attributed to the secondary metabolites it produces. This overview aims to provide a basic overview of lemon grass, stressing its therapeutic properties that make it a valuable ingredient in pharmaceutical and diagnostic products (Ranade *et al.,* 2015). Lemon grass and herbal plant products have medical potential because of their phytochemical components, which evoke certain physiological or pathological effects within the human body. *Cymbopogon citratus* has been widely consumed for its medical, cosmetic, and nutritional properties around the world. Tannins, saponins, flavonoids, phenols, anthraquinones, alkaloids, deoxy sugars, and several important oil components have all been found in studies on the herb's phytoconstituents. Secondary active metabolites of some of the additives have also been linked to the plant's numerous pharmacological effects, including its toxicity profile. Despite popular belief that *Cymbopogon citratus* is safe to eat at any dose, new evidence suggests that factors such as bioactive part variation and dose/length of management may have an impact on its toxicological profile. Although *Cymbopogon citratus* has a wide range of healing effects, it should be used with caution by people with kidney and liver diseases, pregnant or lactating women, patients on antiplatelet medication or who have clotting disorders, and in combination with capsules that rely on the cytochrome P450 enzyme machine for their metabolism. High doses and long-term use of *Cymbopogon citratus* tea or decoctions should be avoided, and more research on dose consistency is needed (Ekpenyong *et al.*, 2014).

Table 2.1. Taxonomic details of this herb are as follows:

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida
Order	Poales
Family	Poaceae
Genus	*Cymbopogon*
Species	*Citrates*

Hamza *et al.*, (2009) carried out a study that disc diffusion method was used to examine the antibacterial properties of Lemon grass against a variety of medical and laboratory isolates of bacteria and fungus. Acetone extracts, along with dichloromethane, methane, and hexane, were shown to have the strongest antibacterial activity, according to them. Water extracts, on the other hand, had the least antibacterial efficacy against bacteria and fungus. They also did phytochemical testing and discovered Saponins, Tannins, Alkaloids, and Flavonoids.

Gore *et al.*, (2010) investigated the antihelmintic effectiveness of *Cymbopogon citratus* leaf extracts against *Pheritima posthuma*. As a control, they employed methanolic and aqueous extracts. Piperazine citrate, a popular medicine, was also employed as a control, as was regular saline. Their research focused on the willpower of time spent paralysed as well as time spent dying of worms. They discovered that the methanolic extract of *Cymbopogon citratus* leaves was more antihelmintic than the aqueous extract.

Naik *et al.*, (2010) conducted a study on lemon grass, researchers discovered that its extract was effective against all of the tested species except *P. aeruginosa.* Gram positive bacteria were shown

to be more susceptible to lemon grass oil than Gram negative bacteria, according to their findings. They also discovered that in the Broth Dilution Method, check organisms were found to be inhibited by lemon grass oil at lower doses than in the Agar Diffusion Method.

Hindumathy (2011) investigated the antibacterial properties of *Cymbopogon citratus* alcohol and water extracts, as well as the presence of phytochemical components, were studied. By employing the disc diffusion approach, he used 4-gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris*) and 2-gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) at 4 specified concentrations. His findings revealed that the extracts prevented the organisms from growing faster. He discovered that the extracts minimal inhibitory concentrations against the microorganisms he studied ranged between 150mg/ml and 50mg/ml. He also discovered that alcohol extracts were found to be more powerful than water extracts. He also conducted a photochemical analysis that revealed the presence of alkaloids and phenol, but not cardiac or cyanogenic glycosides.

Ewansiha *et al.*, (2012) Evaluated phytochemicals and antimicrobial efficiency of *Cymbopogon citratus* (lemon grass) against pathogenic microbes using the cold maceration and agar diffusion method. Hexane, chloroform, and methanol were used as extraction solvents. They found imply zones of inhibition of the chloroform leaf and corresponding root extracts against *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Candida albicans* respectively. Extracts of hexane and methanol showed no interest in the check organisms. For chloroform leaf and root extracts, they discovered the minimal inhibitory concentration (MIC) and the matching minimal bactericidal concentration (MBC).They conducted a phytochemical screening on *Cymbopogon citratus* and discovered the presence of Tannins, Flavonoids, Phenols, Carbohydrates, and a potentially hazardous oil in each of the base and leaf portions. Their findings showed that *Cymbopogon citratus*

indicate zones of inhibition demonstrated intermediate antibacterial interest toward microbes while *C. albicans* become resistant.

Maheswari and Euginamala (2013) carried out an antibacterial and antifungal susceptibility test on lemon grass using the well diffusion technique. They also used phytochemical screening to determine which phytoconstituents were present in the plant material. They also underwent FT-IR and HPLC testing in order to identify the useful organisation and analyse the chemicals.

Soares *et al.*, (2013) reviewed that the *Cymbopogon citratus* was tested for antibacterial and antifungal activity against health-care-associated multidrug-resistant strains like *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia,* and their ATCC manipulate lines. Pathogenic *Candida albicans, Candida parapsilosis,* and *Candida tropicalis* were also tested. The essential oil of *Cymbopogon citratus* was found to be efficient against methicillin-resistant *Staphylococcus aureus* and vancomycin resistant *Staphylococcus epidermidis,* according to their findings. They also discovered that *Cymbopogon citratus* is effective against *Candida albicans*, as well as *Candida parapsilosis* and *Candida tropicalis*, which are on the rise**.**

Soares *et al.*, (2013) investigated the bioactive phytochemical content and antioxidant activity of lemon grass leaf extracts produced using various solvents, including (water, methanol and ethanol). All extracts contained tannins, flavonoids, and terpenoids, according to the phytochemicals analysis. Methanolic extracts also contained alkaloids and steroids, according to their research. While ethanolic extracts had far higher antioxidant activity, the fine effects were achieved by using aqueous extracts.

Roriz *et al.*, (2014) carried out the antioxidant activity of *Pterospartum tridentatum* L., *Gomphrena globosa* L., and *Cymbopogon citratus* was investigated using chromatographic and mass spectrometry techniques, as well as free radical scavenging activity, reducing power, and prevention

of lipid peroxidation in brain homogenates. According to their findings, *Cymbopogon citratus* had the best -carotene bleaching and lipid peroxidation inhibitions, whilst *P. Tridentatum* had the best 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity and reducing power, with mostly dihydroflavonol and isoflavone derivatives. Otherwise, the most prevalent phenolic component in G. globosa was kaempferol 3-O-rutinoside, and betacyanins were only found in this sample.

Geetha *et al.*, (2014) examined that the *Cymbopogon citratus* leaves for phytochemical screening and quantification of primary and secondary metabolites such as chlorophyll, carbohydrates, protein, lipids, phenol, tannin, and flavonoids.

Umar (2016) identified using different solvents such as ethanol, chloroform, and acetone, researchers evaluated phytochemical components and antibacterial effectiveness of *Cymbopogon citratus* leaf extracts against bacterial pathogens using cold maceration and agar diffusion methods. Traditional phytochemical qualitative screening for the presence or absence of many secondary metabolites was performed on all extracts. He used the agar diffusion method to assess the plant extracts' susceptibility to *Escherichia coli, Staphylococcus aureus,* and *Salmonella typhi*. As controls, he utilised Tetracycline, Ciprofloxacin, and Erythromycin. Every extract's Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) have been determined in three concentrations: 100 mg/ml, 50 mg/ml, and 25 mg/ml. He measured the antibacterial activity of leaf extracts against *Staphylococcus aureus, Salmonella typhi,* and *Escherichia coli* using mean area of inhibition.

Elhassan *et al.*, (2016) evaluated the vital oils from three fragrant herbs belonging to the Genus *Cymbopogon* of the Poaceae family, such as *Cymbopogon citratus* (leaves), *C. nervatus* (inflorescences), and *C. proximus*, were studied in vitro for their anti-tubercular properties (leaves and inflorescences). They tested essential oils in vitro for antimicrobial activity against nine medical

isolates and a reference susceptible strain (H37Rv) of M. tuberculosis. They used Lowenstein-Jensen (LJ) medium containing glycerol, at concentrations of 75-15 µl/mL. They assessed antitubercular activity using a method expressed in terms of the minimum inhibitory concentration (MIC).They looked at essential oils from the genus *Cymbopogon* that showed anti tubercular activity in Lowenstein Jensen medium against all of the M. tuberculosis isolates tested, at a concentration of 15 l/ml for each oil. Rifampicin resistance was confirmed in some lines.

Olayemi (2017) carried out a study to determine the root, stem, and leaf crucial oils of *Cymbopogon citratus* cultivated in Kaduna, North Central Nigeria, were studied and extracted one at a time using hydro-distillation and GC-MS. The chemical makeup of the oils was analysed, revealing the identities of 34, 26 and 16 chemicals, respectively. The isomers geranial and neral, which together shape the compound citral, have been the principal additions in three oils. This translates to 35.1 percent of the chemicals detected inside the root, stalk, and leaf oils, respectively.

Oluyemi, *et al.*, (2018) conducted that it was discovered that the methanol extract of lemon grass yielded the greatest percentage yield of 10.3%, but tannin and alkaloid were found in all extracts. In human volunteers and rats, DEET (N, N-diethyl-3 methylbenzamide) had 100% repellency to Anopheles mosquitoes for 300 minutes after application. In human volunteers, water, chloroform, and methanol extracts had percentage repellency reduced from 100% to 94 percent after 60 minutes, 100% to 94 percent after 120 minutes, and 100% to 83 percent after 150 minutes. Water, chloroform, and methanol extracts diminish percentage repellency in Swiss albino rats from 100% to 87 percent after 90 minutes, 100% to 87 percent after 150 minutes, and 100% to 90% after 180 minutes after treatment, respectively.

Nyamath & Karthikeyan (2018) Screened for antimicrobial activity against *Bacillus vallismortis, Lysinibacillus macroides, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and

Vibrio cholera at three different concentrations using the agar well method on extracts of lemon grass leaves prepared in solvents such as ethanol and methanol. Lemon grass extract was reported to have potent antibacterial properties against *Staphylococcus aureus*, with a zone of inhibition (12.50mm) at 1,000 ppm. At 250 ppm, the least zone of inhibition was identified in *Pseudomonas aeruginosa* (2.0mm), indicating that the lemon grass leaves extract had a strong antibacterial activity against antibiotic-resistant bacteria.

Gupta *et al.*, (2019) examined Thin Layer Chromatography was used to analyse the phytochemical screening of ethyl acetate extract of lemon grass leaves, as well as the qualitative evaluation. They tested phytochemicals for the presence of a variety of bioactive components such as flavonoids, phenols, tannins, and alkaloids, among others. TLC analysis revealed the existence of these secondary metabolites as well. The antibacterial activity of the ethyl acetate extract against *Bacillus subtilis* was established.

Bhardwaj (2020) screened secondary metabolites such as tannins, flavonoids, phenolics, saponins, steroids, cardiac glycosides, and alkaloids were discovered using qualitative techniques and plant screening. Plants with these phytochemicals and secondary metabolites have a higher therapeutic potential, according to him**.**

Guleria *et al.*, (2020) conducted to determine the general phenolic content and antioxidant ability of lemon grass tea made from fresh and dried leaves. In many antioxidant models, such as DPPH (10.02 and 9.54mg/ml), ABTS (97 and 10.78mg/ml), nitrite radicals (12.38 and 11.30mg/ml), and anti-lipid peroxidation (13 and 1.01mg/ml), the EC50 values of fresh and dried lemon grass extracts were shown to be equivalent. They also looked at the phytochemicals and discovered that fresh and dried lemon grass leaves have TPC (28.1 and 32.1 mg gallic acid equivalent/g) and flavonoid content material (47 mg QE/g and 14.6 mg QE/g), respectively. They discovered that the antioxidant

capacity of fresh and dried leaves is nearly identical.

Lin *et al.*, (2020) studied *Cymbopogon citratus* was tested for antibacterial activity against uropathogens isolated from UTI patients, including *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli.* They used the disc diffusion technique to analyse an ethanolic extract of *Cymbopogon citratus*, and the least inhibitory concentration was discovered and quantified. They also used HPLC to characterise the phytochemical components found in *Cymbopogon citratus* ethanolic extract. They tested antibacterial susceptibility by measuring zone of inhibition (ZOI), and found that *E. coli, S. aureus,* and *P. aeruginosa* showed no zone whereas *K. pneumoniae* displayed an 8mm zone in opposition to ethanolic extract. The flavonoids and phenolics additives in the ethanolic extract of *Cymbopogon citratus* were validated by HPLC profiling. *Cymbopogon citratus* also reduced a wide range of uropathogens in the mouse version**.**

Chapter-3 MATERIAL AND METHODS

The present investigation was carried out in Department of Cell Biology, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, U.P., India during 2019-20 to study on phytochemical profiling and antimicrobial activity of *Cymbopogon citratus* (Lemon grass).

3.1 Chemicals, Equipments and Miscellaneous Items

All chemicals, equipment, laboratory wares and miscellaneous items used for the study are enlisted in annexure-I, while the details of chemicals are given in annexure-II.

3.2 Sterilization

3.2.1 Sterilization of glasswares

Glasswares were boiled in detergent solution, cool and again wash under tap water, and rinsed with distilled water. Stained glasswares were washed with Chromic acid (20 to 30 gm potassium dichromate, 0.1% sulphuric acid) and rinsed with distilled water. After washing, the glasswares were autoclaved at 121^{o}C, 15 psi for 40 to 50 minutes. Before using, all glasswares were kept in hot air oven for dry sterilization at 160^{o}C for 30 to 40 minutes.

3.2.2 Sterilization of media

Prior to dispensing, prepared media were autoclaved at 121^{o}C and 15 psi pressure for 30 to 40 minutes for sterilization of media.

3.2.3 Sterilization of working surface area

Laminar air flow cabinet is the working surface area as it provides the aseptic conditions. Firstly, the required instruments were kept in chamber and exposed to UV light for 20 to 25 minutes. Then, wiped with spirit or 70% ethyl alcohol before the pouring of autoclaved media in autoclaved petri plates and the inoculation of samples. All the operation i.e. pouring, streaking, transfer, inoculation etc. were done over a Bunsen burner.

3.3 Isolation of *Cymbopogon citratus* isolates from plant sample.

3.3.1 Collection of plant material

Leaves of *Cymbopogon citratus* were collected from horticultural research Centre, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut- 250110. The leaves were washed thoroughly with tap water to remove any dirt or dust particles and then washed with 70% ethanol for disinfection (for 1 to 3 minutes) then washed with distilled

water (3 times). Then leaves were dried under shaded condition at room temperature and ground into fine powder using mortar and pestle, then stored at 4°C in light air container bottle.

3.3.2 Extract Preparation

2.5-gram powdered leaf of *Cymbopogon citratus* was dissolved in 50ml of different extracts i.e. methanol, ethanol, acetone and distilled water for 2 days with intermediate shaking at regular intervals.

3.3.3 Filtration and Evaporation

The fresh leaves were air dried for four weeks in the absence of contaminations until fully crispy. The leaves were crushed and pounded using clean mortar and pestle and pulverized into fine powder by blender. The powdered leaves were kept in an airtight container to avoid the absorption of moisture. The powdered sample was soaked for 72 hours in 70% methanol, chloroform and aqueous water in the ratio 1:10 each (300g of the powdered sample in 3000 millimeter of 70% methanol, chloroform and water) as solvents to extract the bioactive compounds, after which the samples were first sieved using muslin cloth and filtrate was filtered using membrane filter of 0.45μm pore size. The filtrates were vaporized to dryness using rotary evaporator and were preserved in a sterile bottle at temperature of 4°C for further use. (Dabur *et al.,* 2004).

3.4 Phytochemical Analysis

Phytochemicals are the chemical compounds which are produced by the plants. They are produced as a result of primary and secondary metabolism in plants. These phytochemicals are usually considered as the research compounds because of the biological activity of the compounds are still under the scientific and experimental study towards the

health effects. Thereby the phytochemical analysis of lemon grass extract was carried out using the standard protocol method. (Dhawan, 2017)

Alkaloids

- **Mayer's Test:** The presence of alkaloids can be detected by adding 1 mL of conc. Hcl to 1 mL of extract and a few drops of Mayer's reagent. A white or green precipitate indicates the presence of alkaloids.

Tannins

- **Braymer's Test:** The presence of tannins is shown by the production of a blue green colour after adding 1 ml of distilled water to 0.5 ml of extract and 1 ml of a 5 percent ferric chloride solution.

Flavonoids

- **Lead Acetate Test**:When 1 mL of extract is mixed with 1 mL of a 10% lead acetate solution, a yellow precipitate forms, indicating the presence of flavonoids.

Phenols

- **Ferric chloride Test:** The presence of phenols is indicated by the formation of a reddish brown precipitate when 1 mL of extract is mixed with 1 mL of 5% ferric chloride solution.

Terpenoids

- **Ferric chloride Test:**When 1 ml of extract is mixed with 2 ml of water and 1 ml of a 10% ferric chloride solution, a strong color is formed, indicating the presence of terp enoids.

Glycosides

- **Keller killiani Test:** Add 2 ml of glacial acetic acid to 1 ml of sample, followed by 2 ml of glacial acetic acid, 1 ml of 5 percent ferric chloride solution, and 1 ml of dilute Hcl, and a brown ring will form at the interface, indicating the presence of cardiac glycosides.

Saponins

- **Foam Test:**Add 1 mL distilled water to 1 mL extract and vigorously shake; the prese nce of saponins is indicated by the formation of foam.

Test for quinones

- When 1 mL of extract is mixed with 0.5 mL of con Hcl, a yellow precipitate appears, indicating the presence of quinones.

Test for coumarins

- The presence of coumarins is indicated by the production of a yellow colour when 1.5 ml of extract is mixed with 1.5 ml of 10% NaOH.

3.5 Test Microorganisms

The test microorganisms used for antimicrobial analysis were molecularly characterized strains of bacteria and fungus brought from Deptt of Microbiology, CCS University, Meerut. The microbial strains were maintained on Nutrient Broth (NB) at the laboratory of Cell Biology Department, College of Biotechnology, SVPUAT, Modipuram, Meerut. For sub culturing of microbial strains 10 µl of inoculum was transferred into culture tube containing 50 ml of Nutrient broth (Hi-media, India) and incubated at 37˚C for 24 to 48 hours in aerobic incubator and referred to as seeded broth. The freshly grown culture was used for antimicrobial activity.

Table 3.1. Molecular characterized Bacteria and Fungi species.

Bacterial Species	
Species	**Code**
Bacillus cereus	CCS B 133
Pseudomonas aeruginosa	CCS B 159
Bacillus subtilis	CCS B 330
Staphylococcus epidermidis	CCS B 359
Escherichia coli	CCS B 367

Fungi Species

Species	**Code**
Aspergillus fumigatus	CCS F 163
Penicillin chrysogenum	CCS F 261
Candida albicans	CCS F 179

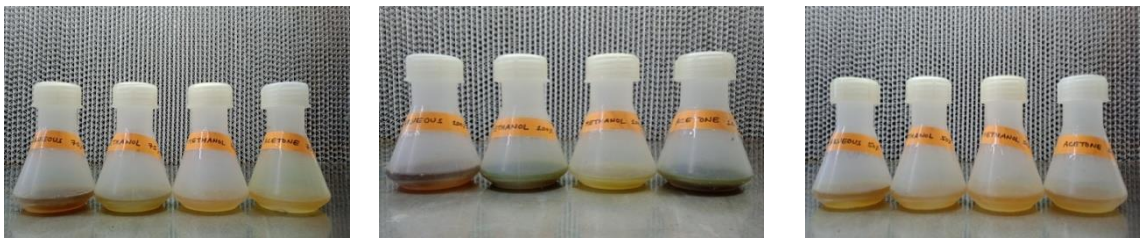


Fig 1. Types of *Cymbopogon citratus* leaf extract used with different percentage (50%, 75%, 100%).

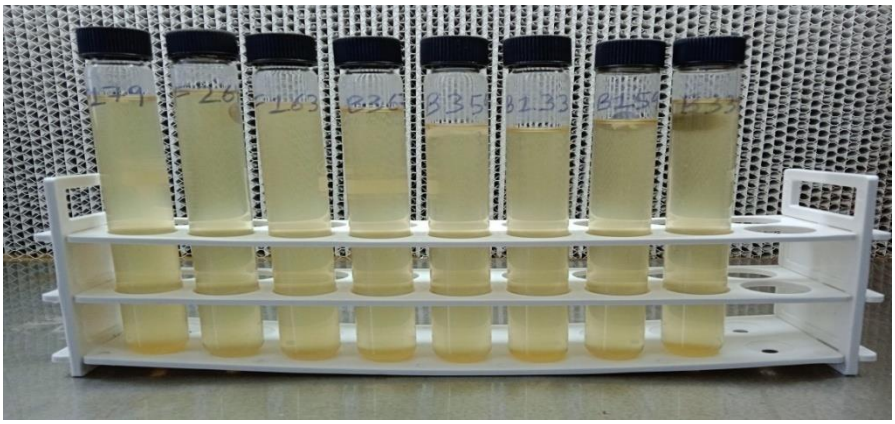


Fig 2. Microbial culture inoculated in nutrient broth

3.6 Antimicrobial activity assay

The different extracts were screened for their antimicrobial activity by well diffusion method, and a standard was prepared and tested to compare the antimicrobial activity of plant extract against both bacterial and fungi strains. The lawn culture of test organism from seeded broth was inoculated with the help of sterilized cotton swabs on solid culture media which was used for well diffusion methods. Muller Hinton Agar media was used for antibacterial activity, whereas Sabouraud Dextrose Agar media was used for antifungal activity. After inoculation, wells of 8 mm diameter were made in the culture plates with the help of sterile cork borer. 50 µl leaf extract of *Cymbopogon citratus* with a concentration of (20 mg/ml) was added into the well and allowed to diffuse in the agar medium. The plates were incubated in aerobic incubator at 37˚C for 24 hours. The antimicrobial activity of the extract was determined by measuring the diameter of zone of inhibition. For each bacterial and fungal strain, controls were maintained of pure solvents without extracts.

3.7 Determination of MIC (Minimum inhibitory concentration)

3.7.1 Agar macro-dilution method

MIC is defined as the lowest concentration of extract in which no growth was observed

after incubation. Four different extracts i.e. Methanol extract, Ethanol extract, Acetone extract and Aqueous extract with a concentration of 100% (Pure extract), 75% and 50% (Aqueous extract) were used for determination of MIC. For making 75% extract, a ratio of DMSO: Distilled water (75 : 35) was added in pure solvents and for 50% extract a ratio of (50 : 50) of (DMSO: Distilled water) was used. The growth inhibition of bacteria and fungus mentioned above was calculated as the percentage of inhibition of the radial growth relative to the standard by following formula:

$$\text{Percentage of inhibition (\%)} = \frac{\text{1-Radical growth of treatment (mm)}}{\text{Radical growth of standard (mm)}} \text{ X 100}$$

3.7.2 Antibiotic Susceptibility method

To compare the antimicrobial activity of different extracts of *Cymbopogon citratus*, certain commercially available antibiotic discs of Ketoconazole (Vandepette *et al.*, 1991), Chloramphenicol (Habiba *et al.*, 2021), Ciprofloxacin (Bauer *et al.*, 1966) and Penicillin-G (NCBI) with a concentration of 30 mcg each were tested to make standard. These antibiotic discs were tested against each bacterial strain on Muller Hinton Agar media as well as against each fungal strain on Sabouraud dextrose agar media. Each antibiotic disc was placed on inoculated culture media with the help of sterilized forceps prior to the antimicrobial testing of lemon grass extracts.

Chapter -4 EXPERIMENTAL RESULTS

In this chapter, outcomes of the present study "A study on phytochemical profiling and antimicrobial activity of *Cymbopogon citratus* (Lemon grass)" from different samples has been described which was carried out at the Department of Cell Biology, College of Biotechnology, Sardar Vallabhbhai Patel University of Agriculture & Technology, Modipuram, Meerut, during 2018-2020.

4.1 Phytochemical Analysis

4.1.1 Effects of solvent on extraction yield of phytochemicals of *Cymbopogon citratus*

The percentage yield of methanol, ethanol, acetone and aqueous extracts of *Cymbopogon citratus* is presented in Table 4.1. In present study, the extraction yield was found to be ranged

from 29 to 84%.

$$\text{Percentage Yield \%} = \frac{\text{Weight of product after evaporation}}{\text{Weight of powder used}} \times 100$$

Table 4.1. Percentage Yield of different leaf extracts of *Cymbopogon citratus*.

Type of Solvent Used for extraction	Percentage Yield at different Concentrations		
	50%	75%	100%
Methanol	54	76	78
Ethanol	73	74	78
Acetone	29	76	84
Aqueous	71	76	74

In methanol extract, the highest percent of yield was found to be at 100% concentration and lowest percent of yield was found to be at 50% concentration. In ethanol the highest percent of yield was found to be at 100% concentration and lowest percent of yield was found to be at 50% concentration. In acetone extract, the highest percent of yield was found to be at 100% concentration and lowest percent of yield was found to be at 50% concentration. In aqueous extract the highest percent of yield was found to be at 75% concentration and lowest percent of yield was found to be at 50% concentration.

4.1.2 Qualitative Phytochemical Analysis of *Cymbopogon citratus* leaf extracts.

The Phytochemical analysis of lemon grass leaf extracts using Methanol, Ethanol, Acetone and Aqueous Extract was showed in Table 4.2. and Fig 3. respectively. All the extracts were used at 100 % concentration.

In methanol extract, it was observed that the phytochemicals (Tannins, Flavonoids,

Glycosides, Terpenoids, Quinones, Coumarins) were found to be present while Alkaloids, Phenols and Saponins were absent. In ethanol extract, it was observed that the phytochemicals (Alkaloids, Tannins, Glycosides, Phenols, Flavonoids, Terpenoids, Coumarins and Saponins) were present while Quinones were absent. In acetone extract, it was observed that (Alkaloids, Phenols, Glycosides, Terpenoids and Coumarins) were present while Flavonoids, Tannins, Saponins and Quinones were absent. In aqueous extract (Flavonoids, Phenols, Glycosides, Terpenoids, Coumarins and Saponins) were present while Alkaloids, Tannins and Quinones were absent.

Table 4.2. Qualitative Phytochemical Analysis of *Cymbopogon citratus* leaf extracts in different solvents, where Positive sign (+) = Present; Negative sign (-) = absent

Sr.No	Phytochemicals	Name of Test	Methanol	Ethanol	Acetone	Aqueous
1	Alkaloids	Mayer's Test	-	+	+	-
2	Phenols	Ferric chloride Test	-	+	+	+
3	Flavonoids	Lead acetate Test	+	+	-	+
4	Tannins	Braymer's Test	+	+	-	-
5	Saponins	Foam Test	-	+	-	+
6	Glycosides	Keller Killiani Test	+	+	+	+
7	Terpenoids	Ferric chloride Test	+	+	+	+
8	Quinones	-	+	-	-	-
9	Coumarins	-	+	+	+	+

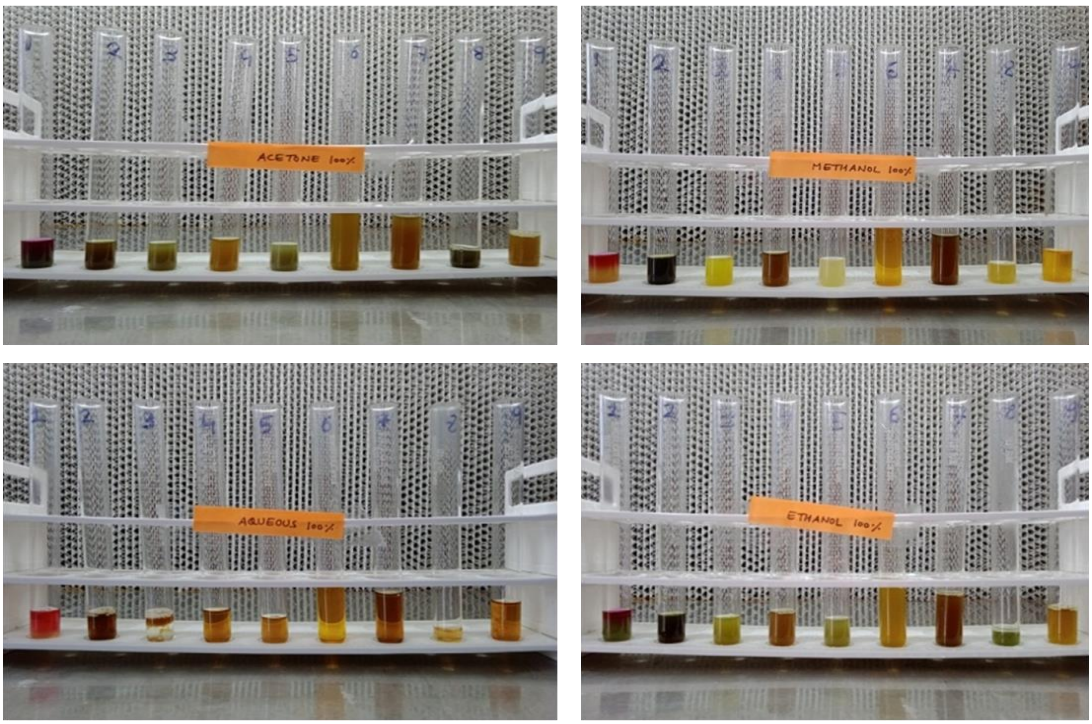


Fig 3. Phytochemical screening of *Cymbopogon citratus* leaf extract in different solvents such as: Methanol (M), Ethanol (E), Acetone (A) and Aqueous (Aq) extract.

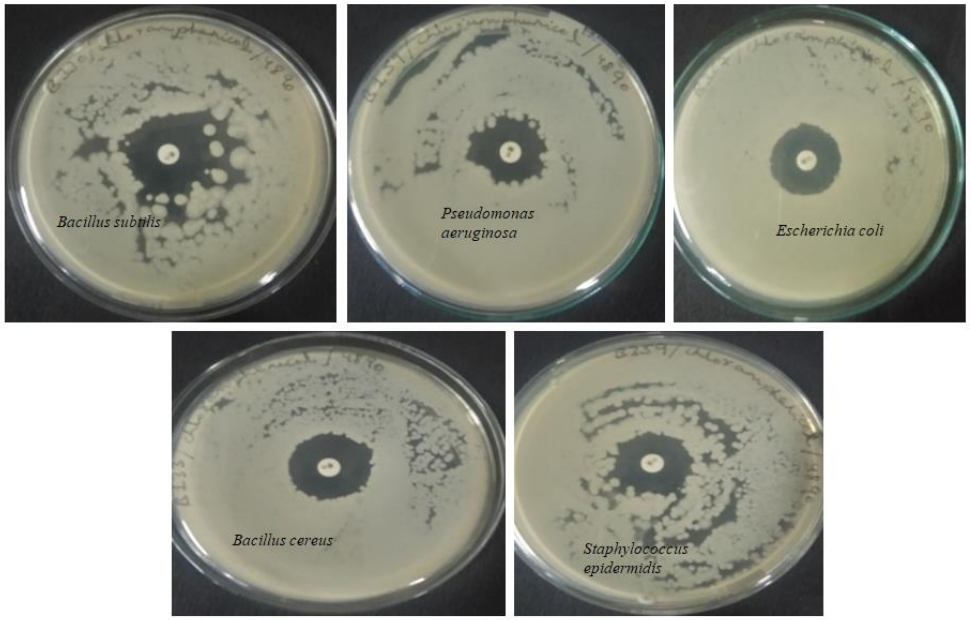


Fig 4. Antibiotic Susceptibility test of Choramphenicol (C) against Bacterial species.

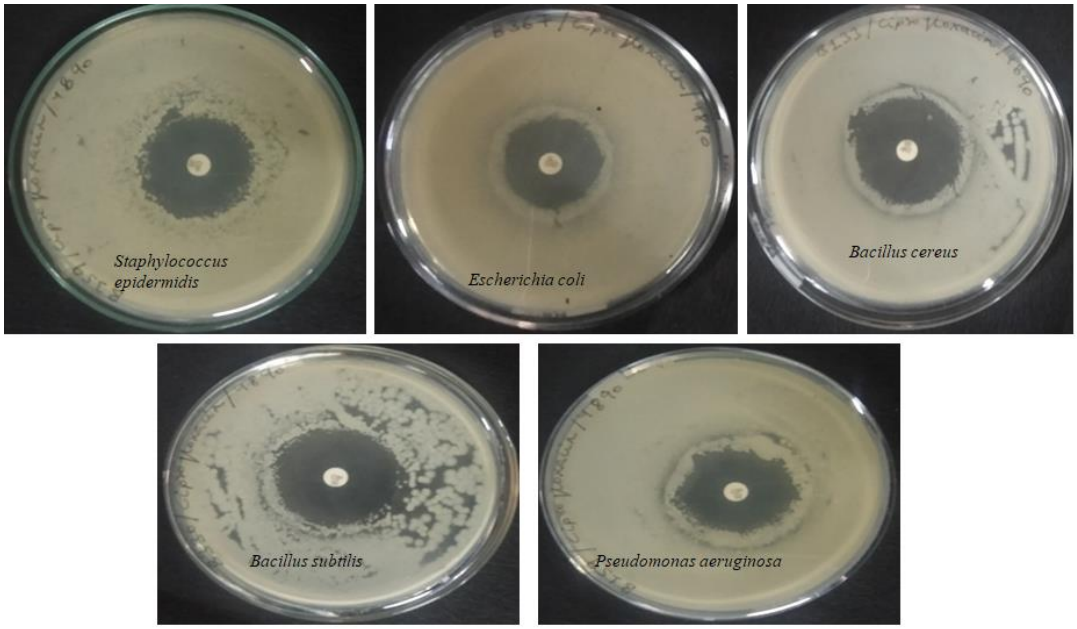


Fig5. Antibiotic Susceptibility Test of Ciprofloxacin (CIP) against Bacterialspecies.

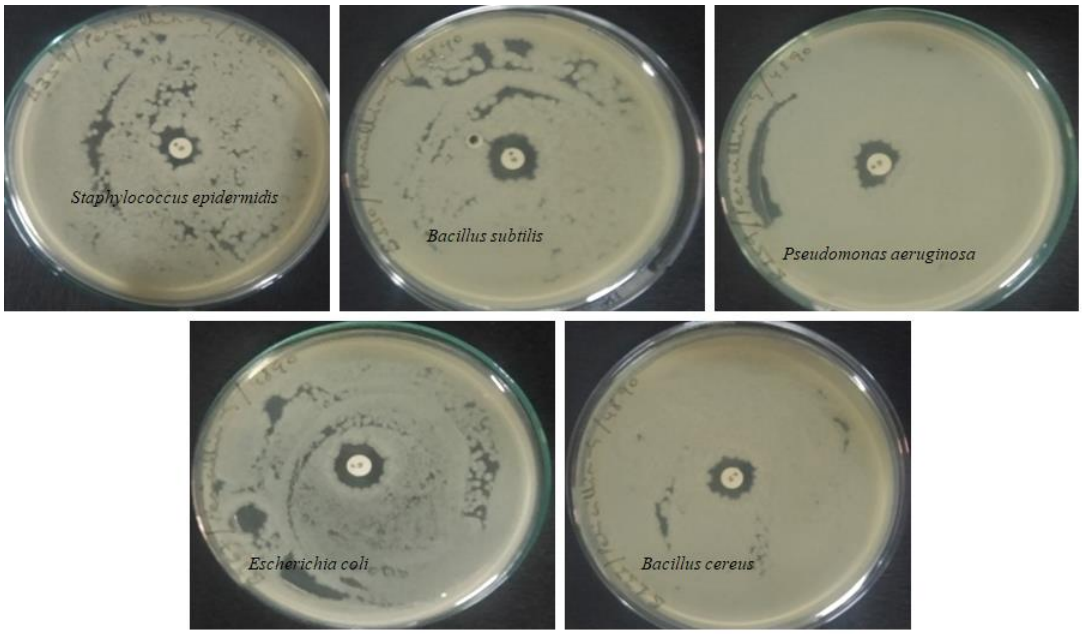


Fig 6. Antibiotic Susceptibility Test of Penicillin-G (P) against Bacterial species.

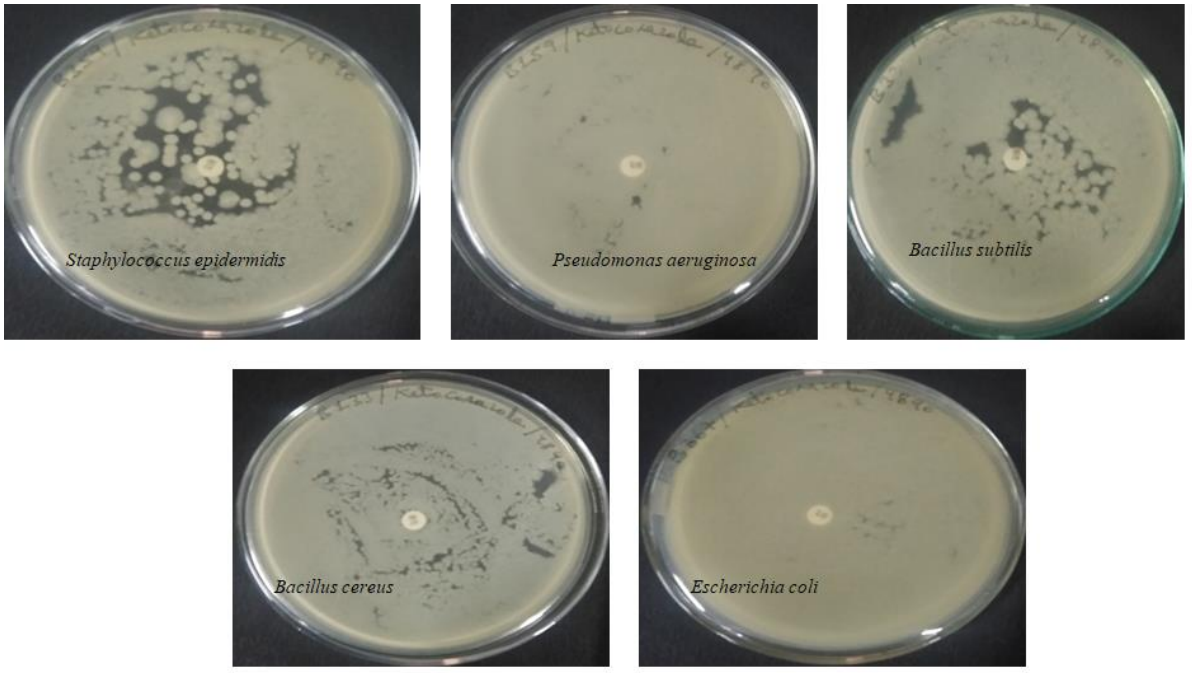


Fig 7. Antibiotic Susceptibility Test of Ketoconazole (K) against Bacterial species.

Fig 8. Antibiotic Susceptibility Test of Ciprofloxacin (CIP) against Fungi species.

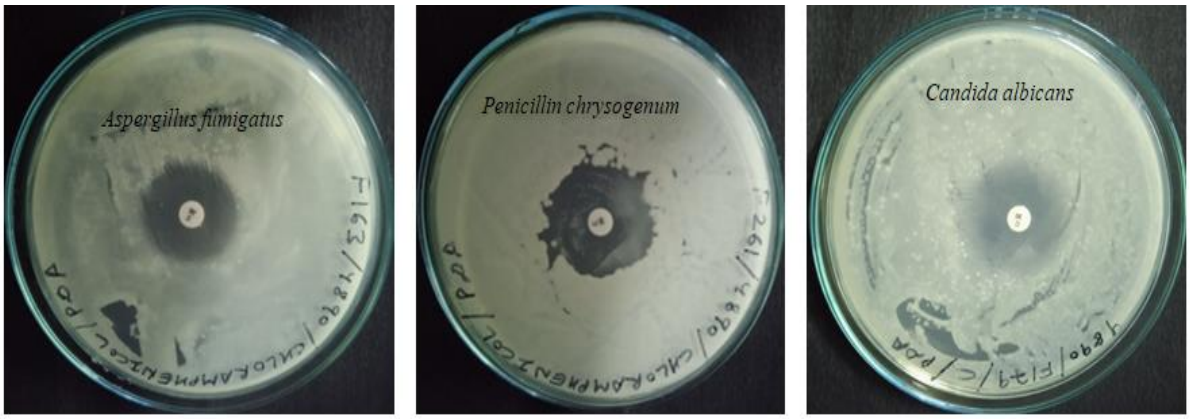


Fig 9. Antibiotic Susceptibility Test of Chloramphenicol (C) against Fungi species.

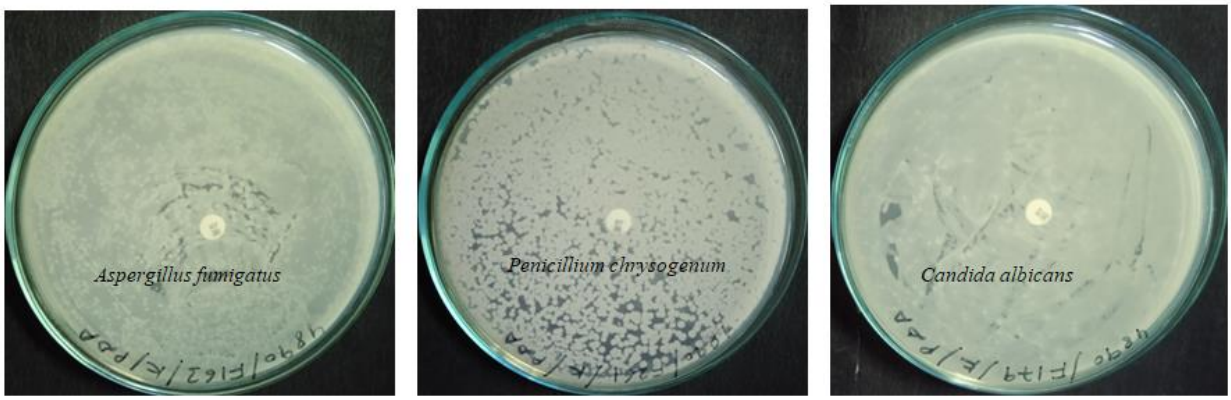


Fig 10. Antibiotic Susceptibility Test of Ketoconazole (K) against Fungi species.

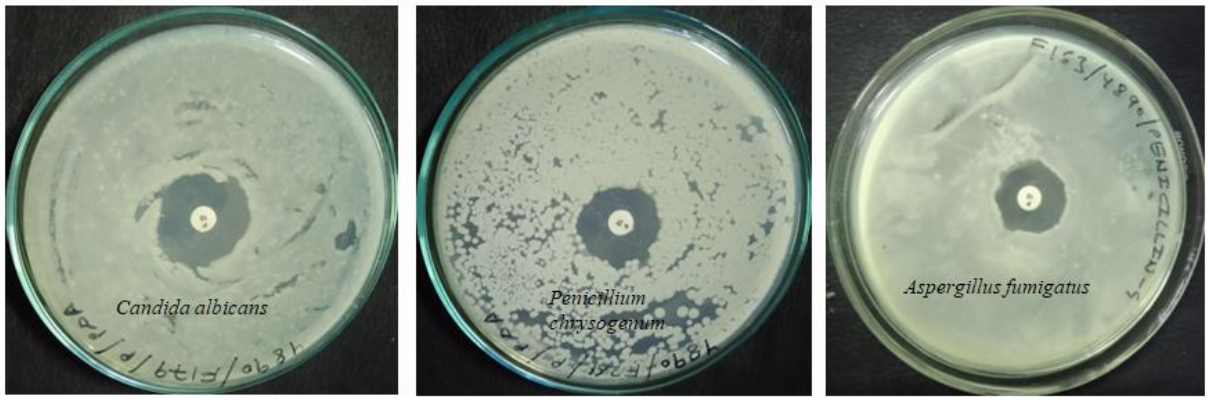


Fig 11. Antibiotic Susceptibility Test of Penicillin-G (P) against Fungi species.

4.2 Analysis of *in vitro* antibiotic susceptibility test

The *in vitro* testing of antibiotic discs against all bacterial and fungal strains showed that ciprofloxacin only showed susceptibility against *Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Aspergillus fumigatus, Candia albicans and penicillium chrysogenum.* Choramphenicol and Penicillin-G showed susceptibility test against all bacterial and fungal strains and Ketoconazole showed no susceptibility test against all bacterial and fungal strains. The zone of inhibition formed by Ciprofloxacin was in the range of 30 to 40mm for bacteria, while the zone of inhibition formed against fungal strains was in the range from 25 to 30mm. The zone of inhibition formed by Chloramphenicol against bacteria was in the range from 22 to 25mm, while against the fungus strains it was ranged from 25 to 28mm. The zone of inhibition formed by Penicillin-G against bacteria was ranged from 10 to 14mm, while it was ranged from 19 to 20mm against fungi strains.

Table 4.3. Antibiotics susceptibility test against Bacterial Species.

S.N	Bacteria	Treatment of antibiotic (Zone of inhibition-mm)			
		Ciprofloxacin	Chloramphenol	Penicillin-G	Ketoconazole
1.	*Bacillus cereus*	36	23	12	-
2.	*Pseudomonas aeruginosa*	35	23	11	-
3.	*Bacillus subtilis*	40	25	14	-
4.	*Staphylococcus epidermidis*	30	25	10	-
5.	*Escherichia coli*	30	22	14	-

Table 4.4. Antibiotics susceptibility test against Fungi Species.

S. No	Fungus	Treatment of antibiotic (Zone of inhibition in mm)			
		Ciprofloxacin	Chloramphenol	Penicillin-G	Ketoconazole
1.	*Aspergillus fumigatus*	30	25	19	-
2.	*Candida albicans*	29	25	19	-
3.	*Penicillium chrysogenum*	25	28	20	-

4.3 Analysis of *in vitro* antimicrobial activity of *Cymbopogon citratus* leaf extract

For analysis of anti-bacterial and anti-fungal activity of *Cymbopogon citratus* leaf extract, four different solvents (methanol, ethanol, acetone and aqueous) were used in different percentage i.e. 50%, 75% and 100% against all Bacteria and Fungi species mentioned above. The antimicrobial activity of Cymbopogon citratus leaf extract was determined on the basis of a clear circular zone or ring, which forms around the sample loaded wells in culture media. That clear zone is called zone of inhibition, which means that it is the maximum ability or efficiency of plant extract to inhibit the growth of microorganisms. So, the antimicrobial activity was calculated on the basis of diameter of circular zone formed around the wells in millimeter. The greater the diameter of zone the greater was the antimicrobial activity.

4.3.1 Antimicrobial activity of methanolic extract

Methanol extract of *Cymbopogon citratus* was evaluated for its anti-bacterial and anti-fungal activity at 25 mg/ml concentration at different percentage against *Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Aspergillus fumigatus, Candida albicans and Penicillium Chrysogenum*. It was found that the methanol extract at 50% showed minimum zone of inhibition against all bacteria and fungus species. While it showed maximum zone of inhibition at 100% against all bacteria and fungus species mentioned above. The zone of inhibition formed by 50% aqueous methanol extract was ranged from 10 to 13mm against all bacteria, while 75% aqueous methanol extract was ranged

from 15 to 22mm against all bacteria, but 100% methanol extract formed a zone of inhibition ranged from 27 to 30mm against all bacteria. Against all fungus species it was found that aqueous methanol extract at 50% showed minimum zone of inhibition which ranged from 14 to 16mm, while 75% aqueous methanol extract was ranged from 21 to 24mm and 100% showed maximum zone of inhibition which ranged from 25 to 26 mm.

4.3.2 Antimicrobial activity of ethanolic extract

Ethanol extract of Cymbopogon citratus was evaluated for its anti-bacterial and anti- fungal activity at 25 mg/ml concentration at different percentage against Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Aspergillus fumigatus, Candida albicans and Penicillium Chrysogenum. It was found that the ethanol extract at 50% showed minimum zone of inhibition against all bacteria and fungus species. While it showed maximum zone of inhibition at 100% against all bacteria and fungus species mentioned above. The zone of inhibition formed by 50% aqueous ethanol extract was ranged from 12 to 13mm against all bacteria, while 75% aqueous ethanol extract was ranged from 17 to 20mm against all bacteria but 100% ethanol extract formed maximum zone of inhibition ranged from 26 to 30mm against all bacteria. Against all fungus species it was found that aqueous ethanol extract at 50% showed minimum zone of inhibition which ranged from 15 to 19mm while at 75% aqueous ethanol extract was ranged from 20 to 24mm and maximum zone of inhibition was formed at 100% which ranged from 25 to 28mm.

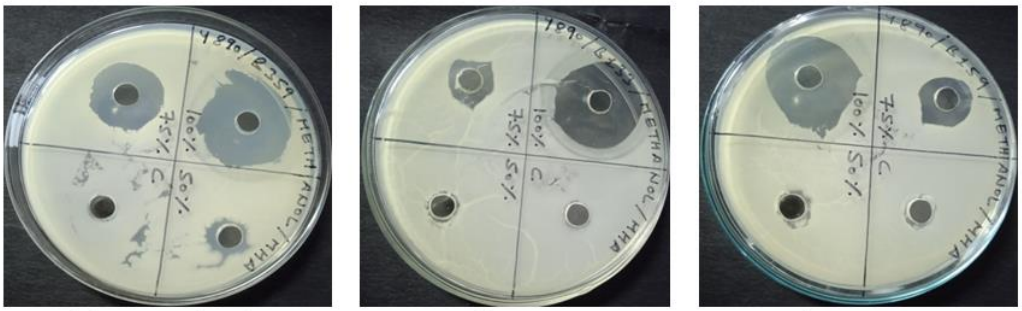
4.3.3 Antimicrobial activity of acetone extract

Acetone extract of *Cymbopogon citratus* was evaluated for its anti-bacterial and anti-fungal activity at 25 mg/ml concentration at different percentage against *Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli,*

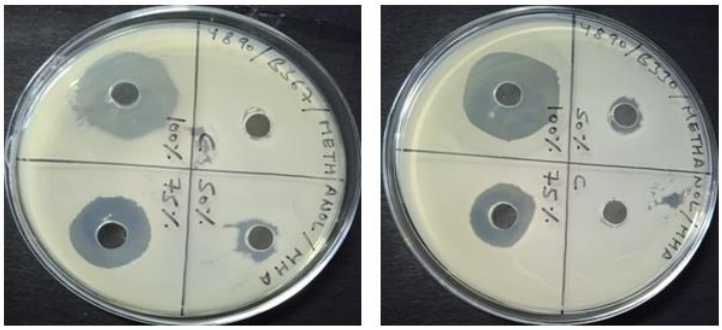
Aspergillus fumigatus, Candida albicans and Penicillium Chrysogenum. It was found that the acetone extract at 50% showed minimum zone of inhibition against all bacteria and fungus species. While it showed maximum zone of inhibition at 100% against all bacteria and fungus species mentioned above. The zone of inhibition formed by 50% aqueous acetone extract was ranged from 9 to 10mm against all bacteria, while 75% aqueous acetone extract was ranged from 18 to 22mm against all bacteria but 100% acetone extract formed a maximum zone of inhibition ranged from 26 to 29mm against all bacteria. Against all fungus species it was found that aqueous acetone extract at 50% showed minimum zone of inhibition which ranged from 15 to 17mm while 75% aqueous acetone extract ranged from 20 to 25mm and maximum zone of inhibition was formed at 100% which ranged from 25 to 27mm.

4.3.4 Antimicrobial activity of aqueous extract

Aqueous extract of *Cymbopogon citratus* was evaluated for its anti-bacterial and anti-fungal activity at 25 mg/ml concentration at different percentage against *Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Aspergillus fumigatus, Candida albicans and Penicillium Chrysogenum*. It was found that the aqueous extract at 50% showed zone of inhibition ranged from 10 to 12mm against *Bacillus cereus, Bacillus subtilis and Staphylococcus epidermidis*. The zone of inhibition formed by 75% aqueous extract was ranged from 19 to 21mm against *Pseudomonas aeruginosa and Escherichia coli*. While aqueous extract showed maximum zone of inhibition ranged from 27 to 29mm at 100% against all bacteria species mentioned above. Against all fungus species it was found that aqueous extract at 50% showed minimum zone of inhibition which ranged from 13 to 20mm while aqueous extract at 75% ranged from 21 to 24mm and maximum zone of inhibition was formed at 100% which ranged from 24 to 26mm.

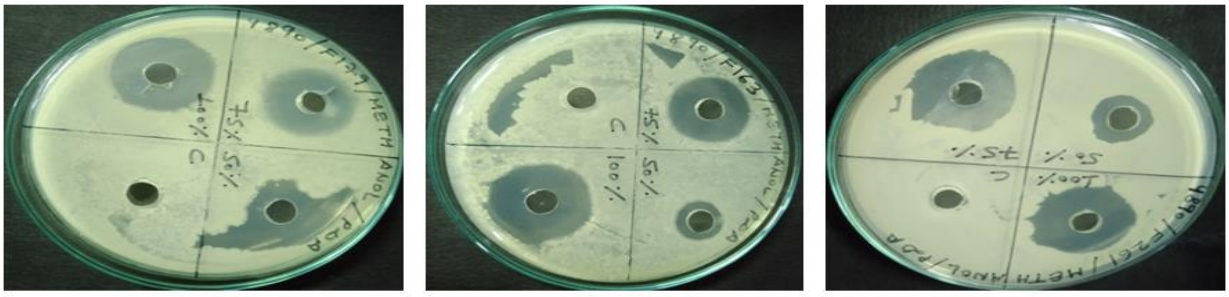


Staphylococcus epidermidis *Bacillus cereus* *Pseudomonas aeruginosa*



Escherichia coli *Bacillus subtilis*

Fig 12. Antibacterial activity of *Cymbopogon citratus* methanolic extract against Bacterial species.



Candida albicans *Aspergillus fumigatus* *Penicillin chrysogenum*

Fig 13. Antifungal activity of *Cymbopogon citratus* methanolic extract against Fungi species.

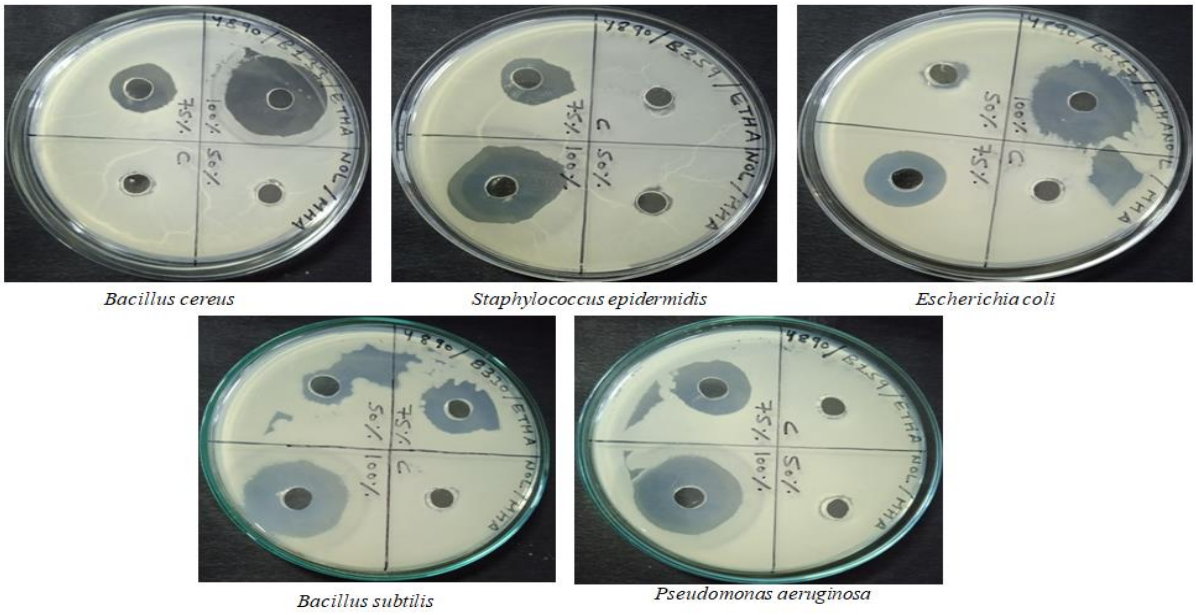


Fig 14. Antibacterial activity of *Cymbopogon citratus* ethanolic extract against Bacterial species.

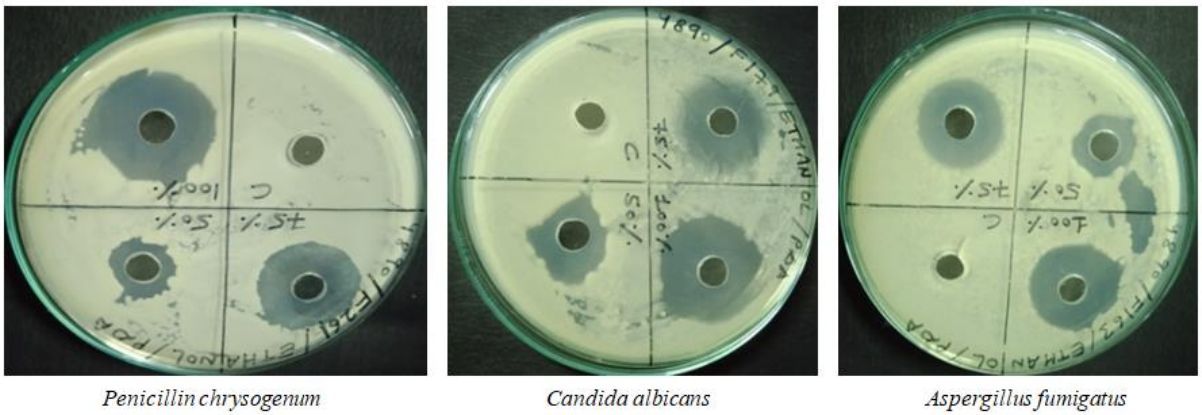
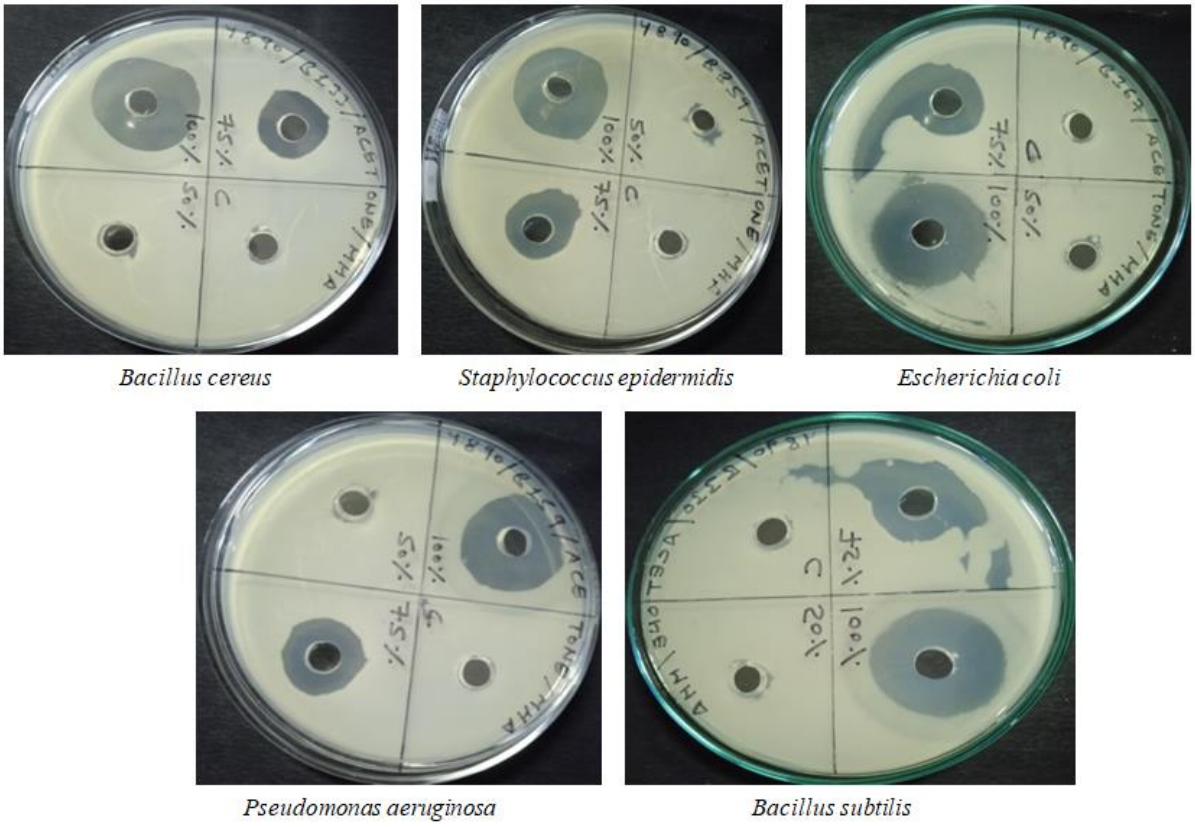


Fig 15. Antifungal activity of *Cymbopogon citratus* ethanolic extract against Fungi species.



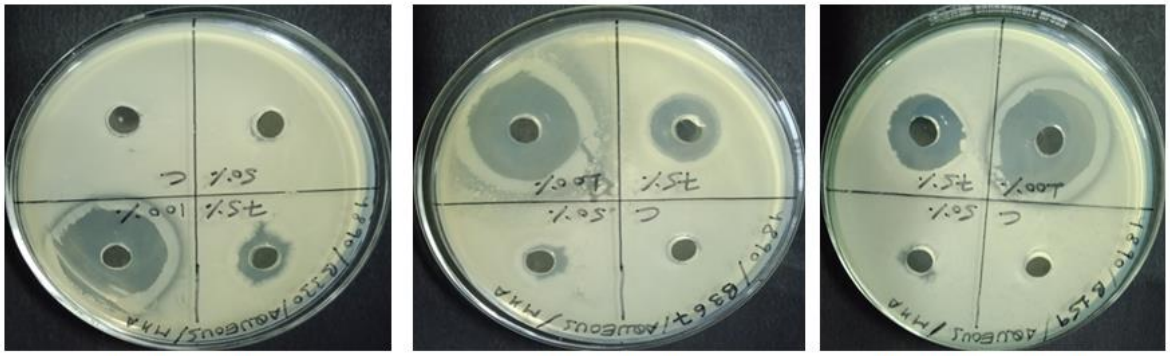
Bacillus cereus *Staphylococcus epidermidis* *Escherichia coli*

Pseudomonas aeruginosa *Bacillus subtilis*

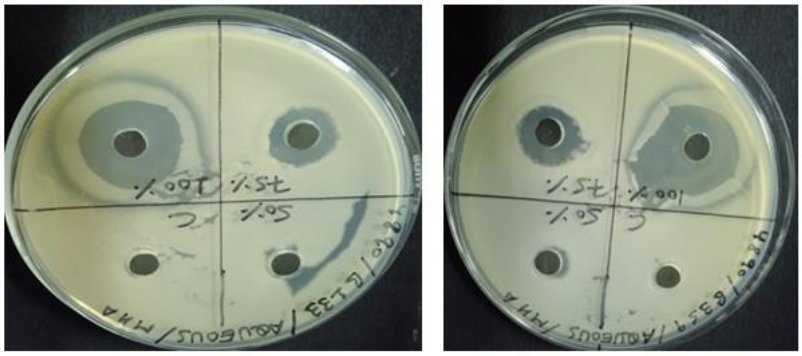
Fig 16. Antibacterial activity of Cymbopogon citratus acetone extract against Bacterial species.

Aspergillus fumigatus *Candida albicans* *Penicillin chrysogenum*

Fig 17. Antifungal activity of *Cymbopogon citratus* acetone extract against Fungi species.

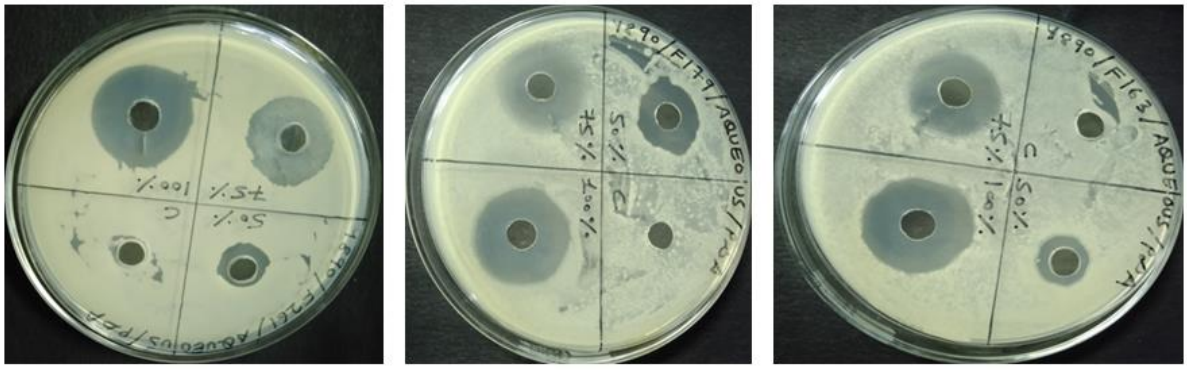


Bacillus subtilis *Escherichia coli* *Pseudomonas aeruginosa*



Bacillus cereus *Staphylococcus epidermidis*

Fig 18. Antibacterial activity of *Cymbopogon citratus* aqueous extract against Bacterial species.



Penicillin chrysogenum *Candida albicans* *Aspergillus fumigatus*

Fig 19. Antifungal activity of *Cymbopogon citratus* aqueous extract against Fungi species.

Table 4.5. Antibacterial activity of *Cymbopogon citratus* methanolic extract.

S.No	Bacteria	Zone of inhibition in (mm), Treatment of methanol extract			
		Ciprofloxacin(30mcg)	At 50 %	At 75 %	At 100 %
1.	*Bacillus cereus*	36	-	15	28
2.	*Pseudomonas aeruginosa*	35	-	17	29
3.	*Bacillus subtilis*	40	10	17	27
4.	*Staphylococcus epidermidis*	30	13	22	30
5.	*Escherichia coli*	30	13	20	27

Table 4.6. Antibacterial activity of *Cymbopogon citratus* ethanolic extract.

S.No	Bacteria	Zone of inhibition in (mm), Treatment of ethanol extract			
		Ciprofloxacin(30mcg)	At 50 %	At 75 %	At 100 %
1.	*Bacillus cereus*	36	-	17	29
2.	*Pseudomonas aeruginosa*	35	-	19	28
3.	*Bacillus subtilis*	40	12	20	26
4.	*Staphylococcus epidermidis*	30	-	17	30
5.	*Escherichia coli*	30	13	20	29

Table 4.7. Antibacterial activity of *Cymbopogon citratus* acetone extract.

S.No	Bacteria	Zone of inhibition in (mm), Treatment of acetone extract			
		Ciprofloxacin (30mcg)	At 50 %	At 75 %	At 100 %
1.	*Bacillus cereus*	36	-	19	27
2.	*Pseudomonas aeruginosa*	35	-	19	26
3.	*Bacillus subtilis*	40	9	19	27
4.	*Staphylococcus epidermidis*	30	-	18	27
5.	*Escherichia coli*	30	10	22	29

Table 4.8. Antibacterial activity of *Cymbopogon citratus* aqueous extract.

S.No	Bacteria	Zone of inhibition in (mm), Treatment of aqueous extract			
		Ciprofloxacin(30mcg)	At 50 %	At 75 %	At 100 %
1.	*Bacillus cereus*	36	10	19	28
2.	*Pseudomonas aeruginosa*	35	-	21	27
3.	*Bacillus subtilis*	40	12	19	27
4.	*Staphylococcus epidermidis*	30	-	20	29
5.	*Escherichia coli*	30	11	20	27

Table 4.9. Antifungal activity of *Cymbopogon citratus* methanolic extract.

S.No	Fungi	Zone of inhibition in (mm), Treatment of methanol extract			
		Ciprofloxacin(30mcg)	At 50 %	At 75 %	At 100 %
1.	*Aspergillus fumigatus*	30	14	23	26
2.	*Candida albicans*	29	14	21	26
3.	*Penicillium Chrysogenum*	25	16	24	25

Table 4.10. Antifungal activity of *Cymbopogon citratus* ethanolic extract.

S.No	Fungi	Zone of inhibition in (mm), Treatment of ethanol extract			
		Ciprofloxacin(30 mcg)	At 50 %	At 75 %	At 100 %
1.	*Aspergillus fumigatus*	30	16	21	25
2.	*Candida albicans*	29	19	20	26
3.	*Penicillium Chrysogenum*	25	15	24	28

Table 4.11. Antifungal activity of *Cymbopogon citratus* acetone extract.

S.No	Fungi	Zone of inhibition in (mm), Treatment of acetone extract			
		Ciprofloxacin(30mcg)	At 50 %	At 75 %	At 100 %
1.	*Aspergillus fumigatus*	30	16	23	25
2.	*Candida albicans*	29	17	20	27
3.	*Penicillium Chrysogenum*	25	15	25	27

Table 4.12. Antifungal activity of *Cymbopogon citratus* aqueous extract.

S.No	Fungi	Zone of inhibition in (mm), Treatment of aqueous extract			
		Ciprofloxacin(30mcg)	At 50 %	At 75 %	At 100 %
1.	*Aspergillus fumigatus*	30	14	22	24
2.	*Candida albicans*	29	20	24	26
3.	*Penicillium Chrysogenum*	25	13	21	25

4.4 Comparative analysis of antibiotic susceptibility against Bacteria and Fungi.

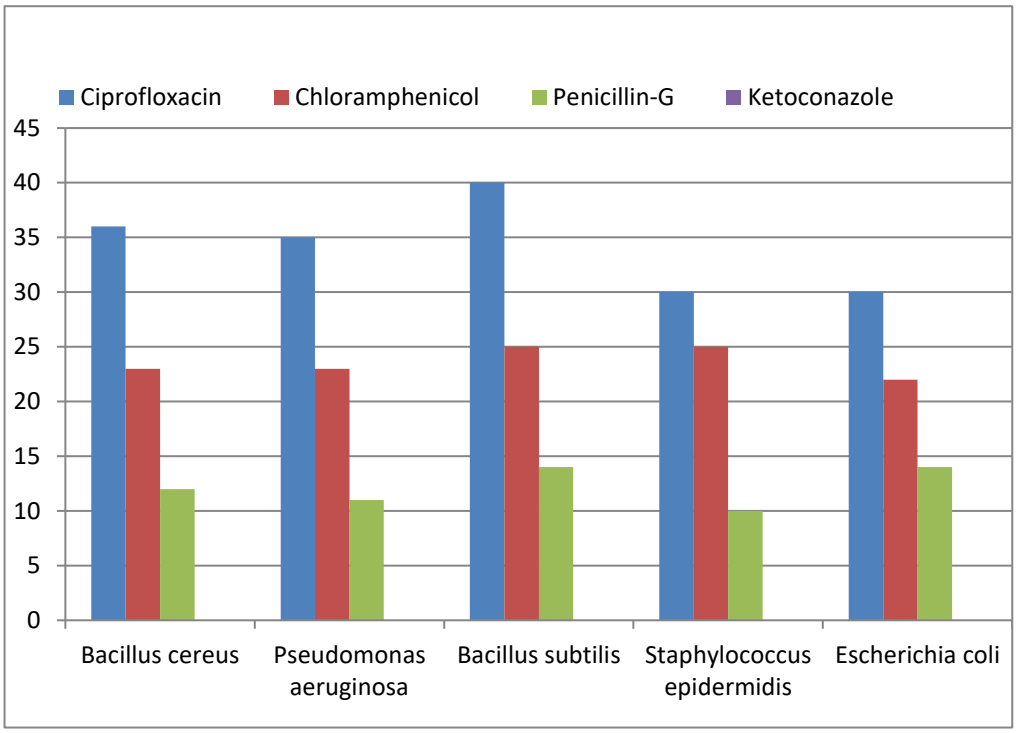


Fig 20. Graphical representation of antibiotics sensitivity for each Bacterial species.

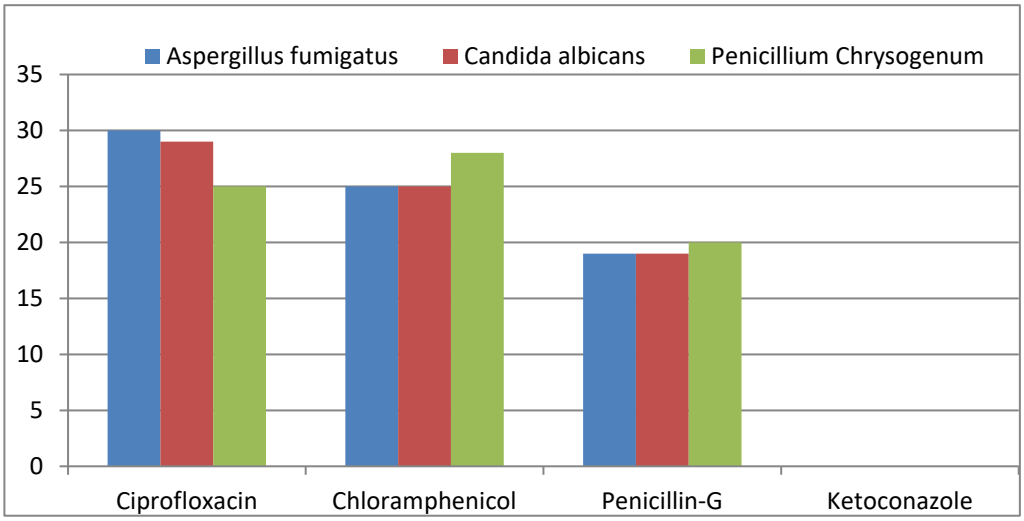


Fig 21. Graphical representation of antibiotics sensitivity for each Fungi species.

4.5 Comparative analysis of different extracts of *Cymbopogon citratus* against Bacterial Species.

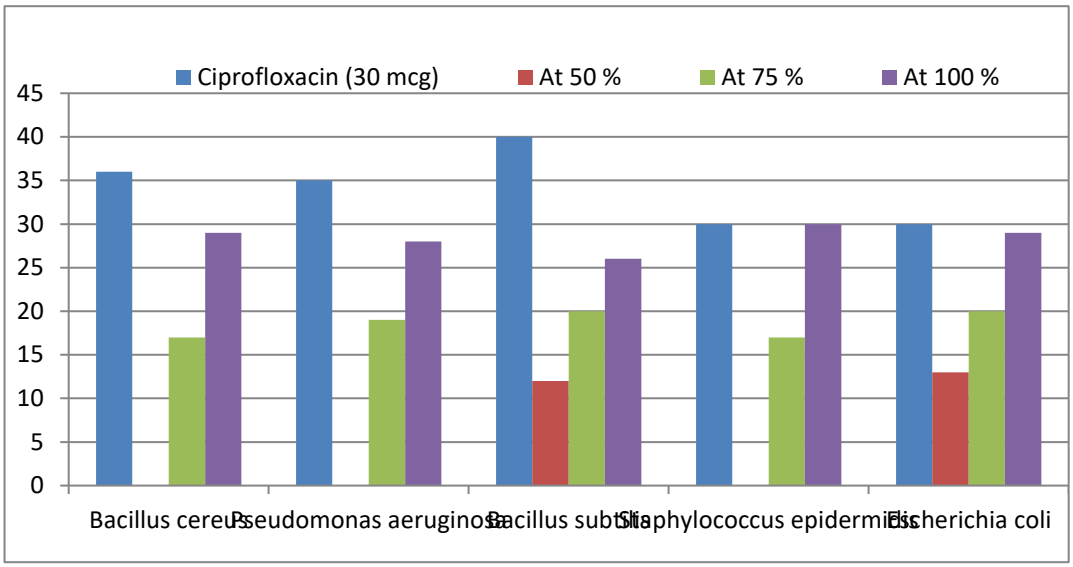


Fig 22. Graphical representation of analysis of Anti-Bacterial activity *Cymbopogon citratus* methanolic extract.

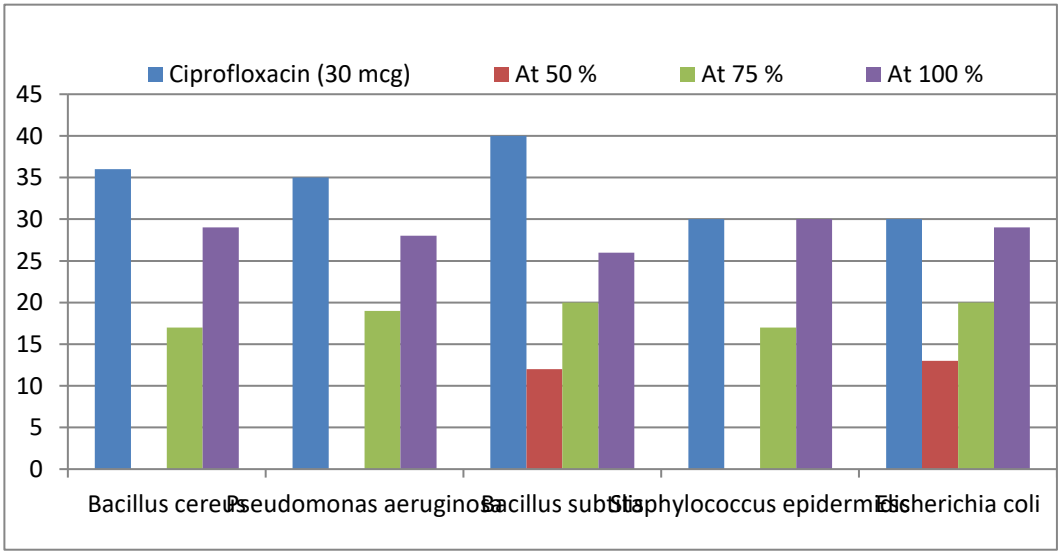


Fig 23. Graphical representation of analysis of Anti-Bacterial activity *Cymbopogon citratus* ethanolic extract.

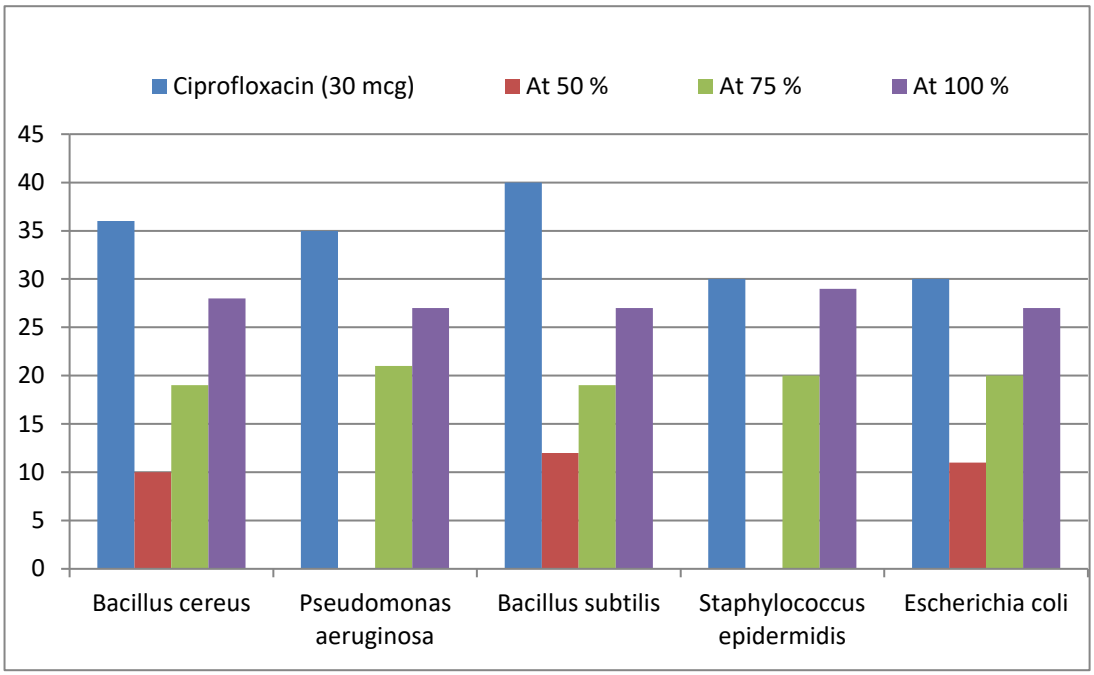


Fig 24. Graphical representation of analysis of Anti-Bacterial activity *Cymbopogon citratus* acetone extract.

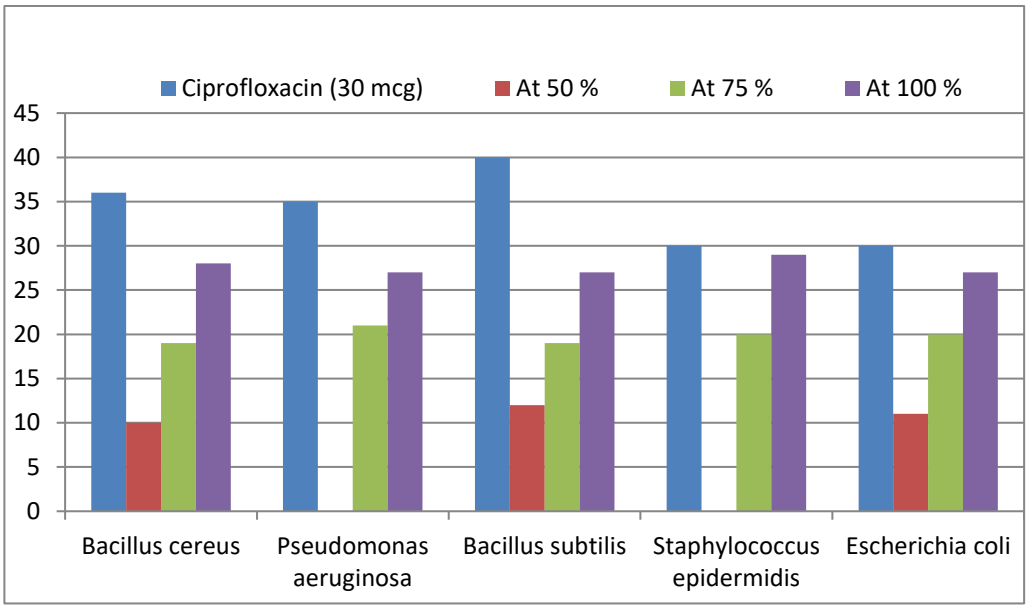


Fig 25. Graphical representation of analysis of Anti-Bacterial activity *Cymbopogon citratus* aqueous extract.

4.6 Comparative analysis of different extracts of *Cymbopogon citratus* against Fungi species.

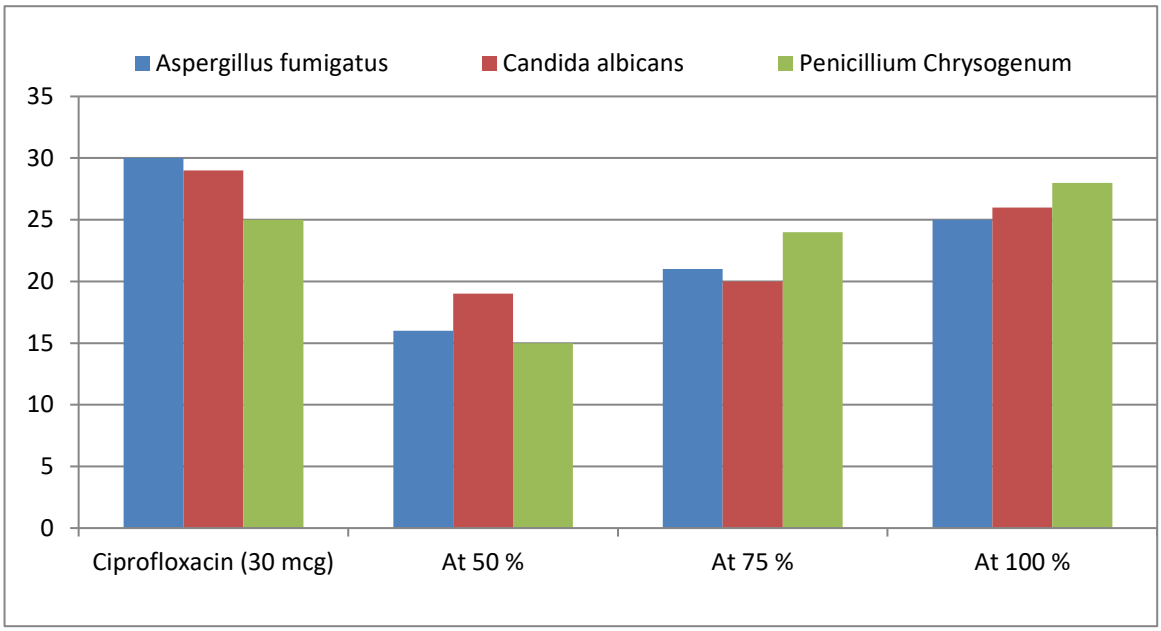


Fig 26.Graphical representation of analysis of Anti-Fungal activity *Cymbopogon citratus* methanolic extract.

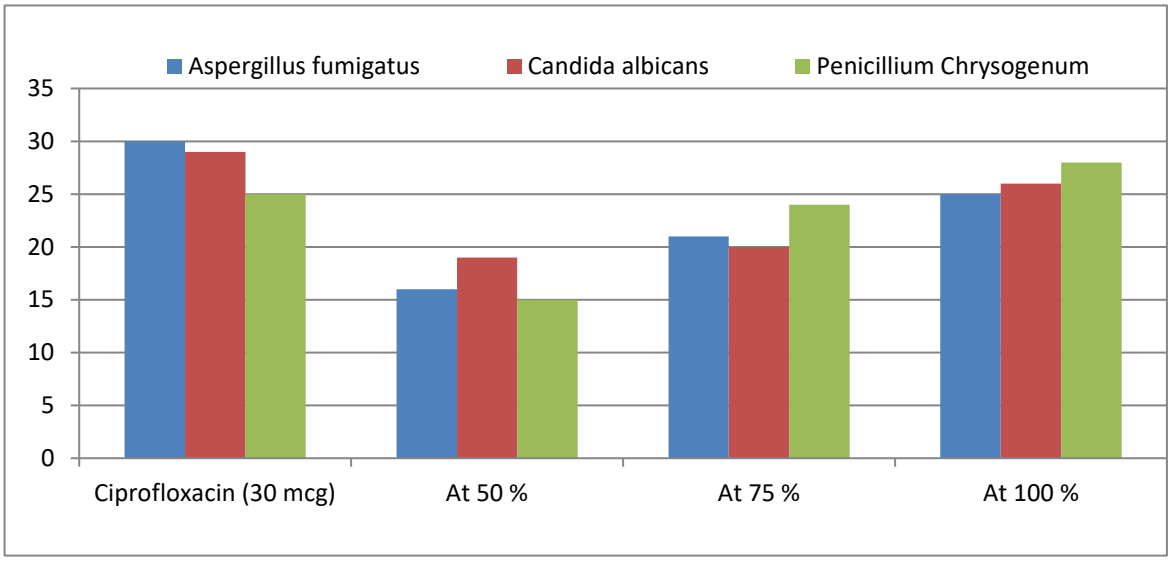


Fig 27.Graphical representation of analysis of Anti-Fungal activity *Cymbopogon citratus* ethanolic extract.

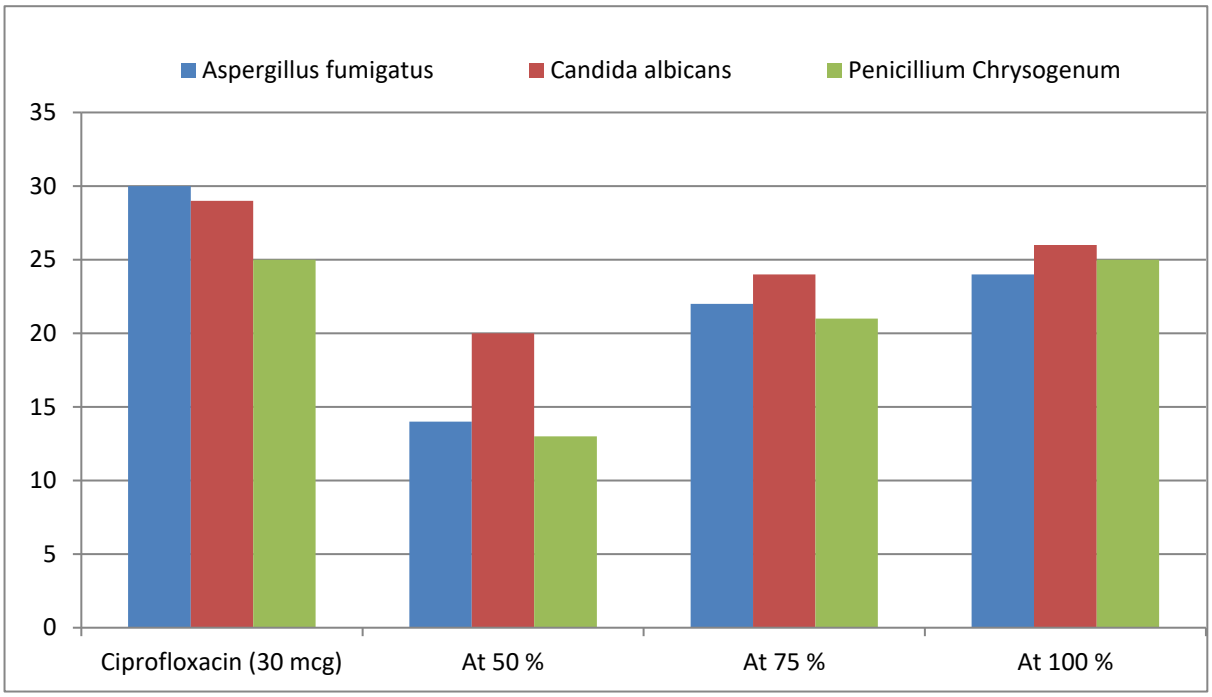


Fig 28.Graphical representation of analysis of Anti-Fungal activity *Cymbopogon citratus* acetone extract.

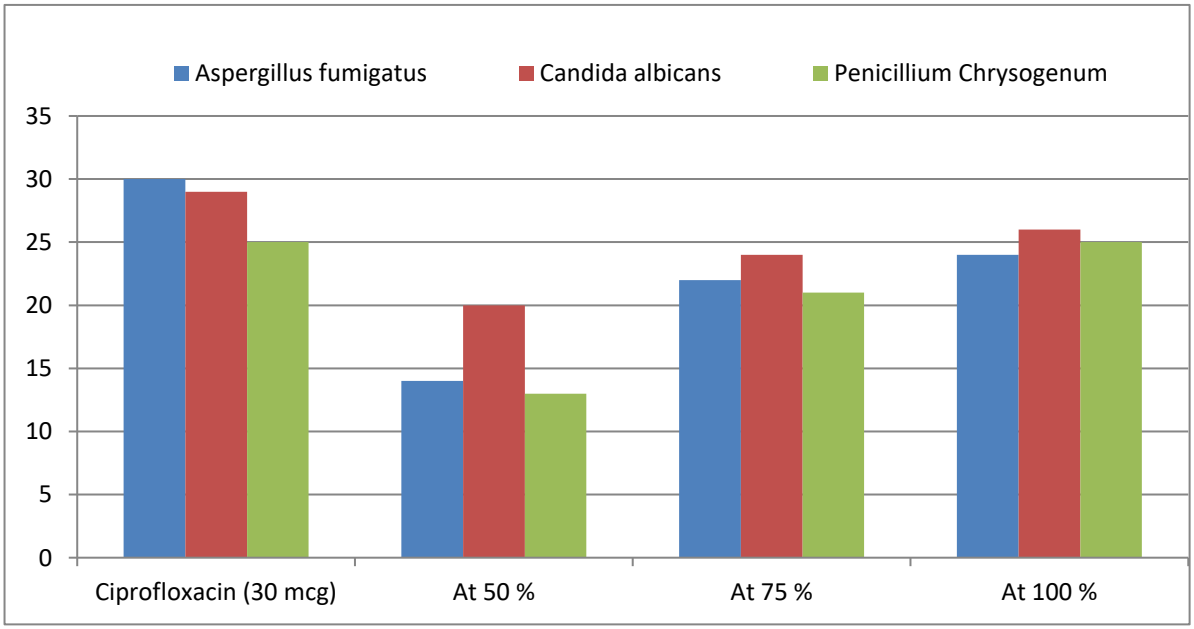


Fig 29. Graphical representation of analysis of Anti-Fungal activity *Cymbopogon citratus* aqueous extract.

CHAPTER 5 DISCUSSION

Cymbopogon citratus commonly known as lemon grass is a well-known aromatic plant which is used for its medicinal purpose, essential oil and an herbal tea, in Ayurveda. It is the most sacred herb, that is useful for the treatment of disorders of Central nervous system (CNS) and also act as an antidepressant. In traditional medicine lemon grass leaves is used for common cough, fever, flu, headache and vascular disorders. The leaves of *Cymbopogon citratus* considered to have natural medicinal properties, including wide array of biological activities such as antioxidant, anti-inflammatory, cardioprotective, anticancer and antimicrobial activity (Ekpenyong *et al.,* 2014) because they contain a number of monoterpenoids (Olayemi, 2017). The antimicrobial activities of plant extract can be attributed to the presence of secondary metabolites.

Therefore, the present study aimed to screen the phytochemical constituents present in *Cymbopogon citratus* leaf which imparts the antimicrobial potential. The Phytochemical analysis was done in different kinds of solvents which can enhance the knowledge for researchers in future. The use of plant extracts and their phytochemicals can be of great significance in therapeutic treatments. Anti-microbial study encourages the use of herbs as alternative or supplementary medicine to reduce the burden of high cost, side effects and progressively increasing drug resistance of pathogens.

5.1 Effect of solvents on percentage yield

Present findings showed that the extraction yield of *Cymbopogon citratus* leaf extracts increased as polarity of the solvent used is decreased. The yield of aqueous solvent extract is lower than that of the pure solvent extracts. These results indicate that increasing the water concentration in the solvent do not enhance extraction yield. The percentage yield of the extract from the solvent extraction showed that methanol solvent gave the highest yield. This may be due to the fact that methanol is a

polar solvent and may be able to extract the active ingredients of the plant without denaturing them. (Oluyemi *et al.,* 2018).

This may be the reason why yields of aqueous methanol, ethanol and acetone extracts are lower than yields of pure extracts. However, extraction yield not only depend on the extraction method but also on the solvent used for extraction. Our findings are in agreement with the extraction yields of some medicinal plants (Adesegun *et al.,* 2013).

5.2 Preliminary screening of phytochemicals

Qualitative phytochemical investigation revealed that the extracts contained some Phytoconstituents such as: alkaloids, phenols, glycosides, terpenoids and coumarins which are present in the acetone extracts. Whereas tannins, glycosides, terpenoids, quinone, coumarins and flavonoids are found in the methanol extracts, also alkaloids and flavonoids were found in aqueous extract. These bioactive components, beside other water-soluble components which are naturally occurring in most plant materials, are known to be bactericidal, pesticidal or fungicidal in nature thus conferring the anti-microbial property of plants (Hamza *et al.,* 2009). Alkaloids, phenols, tannins, flavonoids, glycosides, terpenoids, saponins, coumarins are found in ethanol extracts (Soares *et al.*, 2013) and saponins, phenols, flavonoids, glycosides, terpenoids and coumarins in aqueous extracts. This agreed with the finding of (Hindumathy, 2011).

Thus, the results obtained in the present study indicates lemon grass leaves have the potential to act as a source of useful drugs because of presence of various phytochemical components such as carbohydrate, protein, lipids, phenols, flavonoids and tannin. The results are very much encouraging but scientific validation is necessary before being put into practice (Geetha *et al.,* 2014).

5.3 Comparative analysis of antibiotic susceptibility test

In the present study it was found that all the bacteria species such as *Bacillus subtilis*,

Bacillus cereus, Staphylococcus epidermidis, Escherichia coli and *Pseudomonas aeruginosa* showed highest sensitivity and 0% resistance against ciprofloxacin at 30 mcg concentration our results were in the agreement with the results of (Naik *et al.,* 2010). While all these bacteria species showed moderate sensitivity against chloramphenicol at 30mcg concentration (Habiba *et al*., 2021). However, all the bacteria species showed 100 % resistance against Ketoconazole at 30 mcg concentration (Ewansiha *et al*., 2012).

All the Fungi species such as *Aspergillus fumigatus*, *Candida albicans* and *Penicillium Chrysogenum* showed highest sensitivity against Ciprofloxacin, while all these Fungi species showed moderate sensitivity against Chloramphenicol, however these species showed lowest sensitivity against Ketoconazole. On the basis of our results ciprofloxacin was used as standard for all Bacteria and Fungi species to compare with the antimicrobial activity of *Cymbopogon citratus* leaf extract.

5.4 *In-vitro* Antimicrobial Activity of *Cymbopogon citratus* leaf extract.

For *Bacillus cereus* most effective zone level of (29mm) were observed by ethanol extract at 100% concentration. These results were in the accordance of (Kassahun *et al.,* 2020). For *Pseudomonas aeruginosa* most effective zone levels of (29mm) were observed by methanol extract at 100% concentration. These results were in the agreement with the findings of (Hamza *et al.,* 2009). For *Bacillus subtilis* maximum zone levels of (27mm) were observed by methanol, acetone and aqueous extract at 100% concentration. These findings were in agreement of (Habiba *et al.*, 2021). For *Staphylococcus epidermidis* maximum zone of (30mm) was observed by methanol and ethanol extract at 100% concentration. These results were in the accordance with the results of (Soares *et al.*, 2013). For *Escherichia coli* maximum zone of (29mm) was observed by acetone and ethanol extract at 100% concentration. These results were in the agreement of the findings of (Naik *et al.*, 2010).

For *Aspergillus fumigatus* most effective zone of (30mm) were observed by both methanol and aqueous extracts at 100% concentration. These results were in the accordance of (Dharmagadda *et al.,* 2005) For *Candida albicans* most effective zone of (27mm) were observed by acetone extract at 100% concentration. These findings were in the agreement with the findings of Ewansiha *et al.,* (2012) For *Penicillium Chrysogenum* most effective zone of (24mm) was observed by methanol extract at 100% concentration. These results were also in the accordance of (Dharmagadda *et al.,* 2005).

CHAPTER 6 SUMMARY AND CONCLUSION

The phytochemicals extractable from *Cymbopogon citratus* (lemon grass) is a function of solvents used for extraction and these solvents have varying extractive values. Different solvent-extracts of the same plant have different medicinal and therapeutic usefulness since they contain different bioactive ingredients. Further research can be conducted by examining the anti-microbial activities of the solvent-extracts of lemon grass so that the therapeutic usefulness of these extracts can be established.

The objective of this research was to evaluate the potential of lemon grass leaf extracts on different microbial strains. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.

Phytochemical analysis of Methanol, Ethanol, Acetone and Aqueous extracts of lemon grass revealed presence of alkaloids, flavonoids, steroids, saponins, glycosides, phenols, tannins and terpenoids. The leaf extract of *Cymbopogon citratus* found to be effective against the bacterial species such as *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus epidermidis, Bacillus subtilis* and *Escherichia coli.* It was also found to be effective against the fungal species such as: *Aspergillus fumigatus, Candida albicans* and *Penicillium Chrysogenum*. From the whole study we concluded that the antimicrobial activity of extracts of medicinal plants depends on some parameters like plant material used, technique employed, growth medium and most importantly micro-organism tested. For better research better quality of plant material should be selected. The solvent and the extraction system may both modify the final results. Different extracts of a medicinal plant may show the different results. In vitro testing of *Cymbopogon citratus* shows that the activity of lemon grass leaf extract against all bacteria and fungus was similar than as the standard antibiotic i.e.

Ciprofloxacin. The medicinal value of lemon grass depends upon citral content. The content of citral in lemon grass determines its aroma, quality and its therapeutic utility. Therefore, the citral content of lemon grass is very important economically as well as therapeutically. Medicinal plants are always considered as an effective source of traditional and modern medicines. Many literatures suggest that medicinal and cosmetic importance of lemon grass.

All the extracts evaluated in the present study showed various levels of anti- bacterial and anti-fungal activity and therefore, it was concluded that different secondary metabolites were extracted in different solvents that are responsible for this activity. Another aspect of our antimicrobial study was the dose dependent inhibition of microbial growth. The increase in antibiotic resistance of microorganism to conventional drugs has necessitated the search for new efficient and cost-effective ways for the control of infectious diseases. The results of different antimicrobial and phytochemical studies on *Cymbopogon citratus* and *Cymbopogon flexuosus* form the foundation for the potential source of new anti-microbial agents.

The study was carried out to investigate antimicrobial activity of lemon grass (*Cymbopogon citratus*) leaf extraction the basis of minimum inhibitory concentration (MIC) which is required to control the growth of microorganisms. It was found that for each microbial strain which was tested against each lemon grass leaf extract at different concentration showed different results. The MIC values of each extract was found to be different for all tested microorganisms. For all the tested bacterial species such as *Bacillus cereus,* MIC was found to be (10mm) of aqueous extract at 50% concentration. For *Pseudomonas aeruginosa,* MIC was found to be (17mm) of methanol extract at 75% concentration. For *Staphylococcus epidermidis,* MIC was found to be (13mm) of methanol extract at 50% concentration. For *Bacillus subtilis,* MIC was found to be (9mm) of acetone extract at 50% concentration. For *Escherichia coli,* MIC was found to be (10mm) of acetone extract at 50%

concentration.

For all the tested fungal species such as *Aspergillus fumigatus,* MIC was found to be (14mm) of methanol and aqueous extract at 50% concentration. For *Candida albicans,* MIC was found to be (14mm) of methanol extract at 50% concentration. For *Penicillium Chrysogenum,* MIC was found to be (13mm) of aqueous extract at 50% concentration.

On the basis of the result obtained from the present investigation the following conclusion can be drawn.

- The highest percent of yield was found to be at 100% for all extract and lowest percent of yield was found to be at 50% for all extract by this statement it was concluded that increasing the water concentration in solvent for extract preparation the percent yield decreases.
- All the phytochemicals were found to be present in *Cymbopogon citratus* leaf extract at 100% concentration of solvents because of the highest percent yield.
- In methanol extract, it was observed that most of the phytochemicals (Tannins, Glycosides, Terpenoids, Flavonoids, Quinones and Coumarins) were found to be present at 100% concentration.
- In ethanol extract, it was observed that the phytochemicals (Alkaloids, Tannins, Glycosides, Phenols, Flavonoids, Terpenoids, Saponins and Coumarins) were found to be present at 100% concentration.
- In acetone extract, it was observed that (Alkaloids, Phenols, Terpenoids, Glycosides and Coumarins) were present at 100% concentration.
- In aqueous extract (Flavonoids, Phenols, Glycosides, Saponins, Terpenoids and Coumarins) were present at 100% concentration.
- The methanol extract showed most effective results against *Bacillus subtilis*, *Staphylococcus*

epidermidis, Escherichia coli and *Penicillium chrysogenum* at 50% concentration.

- The ethanol extract showed most effective results against *Bacillus subtilis, Escherichia coli* and *Candida albicans* at 50% concentration.
- The acetone extract showed most effective results against *Bacillus subtilis, Escherichia coli* and *Candida albicans* at 50% concentration.
- The aqueous extract showed most effective results against *Bacillus cereus, Bacillus subtilis, Escherichia coli* and *Candida albicans* at 50% concentration.

The findings of the present study clearly indicate that the leaf extract of *Cymbopogon citratus* plant also possess the anti-microbial activity which may be because of the presence of phytochemicals or secondary metabolites, which has proved evidence for its antimicrobial potential.

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ANNEXURE

Chemicals, Equipments, Lab Ware and Miscellaneous Items

All chemicals used in present study were obtained from Hi-Media as per requirement. Such as Dimethyl Sulfoxide (DMSO), sodium hydroxide (NaOH), potassium acetate, lead acetate, ferric chloride ($FeCl_3$), Lead acetate ($Pb(CH_3COO)_2$) potassium iodide, iodine. Methanol, Ethanol, Acetone, Hydrochloric acid (HCL) nitric acid, sulfuric acid (H_2SO_4), chloroform.

EQUIPMENTS

Microwave Oven

Double distillation apparatus Weighing balance

Hot air oven Laboratory Refrigerator Shaker

Hot plate

Glass wares

Beakers Conical flasks

Measuring cylinders

Reagent bottles

MISCELLANEOUS

Autoclave

Scissors

Cello tape

Tissue paper

Stickers

Blotting paper

Cotton

Whattman filter papers

Marker pens

Cork Bore

Paraffin

CHEMICALS

All reagents/chemicals used for preparation were prepared using autoclaved distilled/MilliQ water.

Reagent preparation for Phytochemical Analysis:

- **Mayer's reagent:** It is used for the detection of alkaloids.
  - Solution (a) 1.36 g of mercuric chloride is dissolved in 60 ml of distilled water.
  - Solution (b) 5 g of potassium iodide is dissolved in 20 ml of distilled water.

- Solution (a) and (b) are mixed and the volume was adjusted to 100 ml with distilled water.

- **Ferric Chloride (alcoholic):** A 5% and 10% w/v solution of ferric chloride in 90% alcohol is used for the detection of phenols.
  - Solution (a): To prepare 5% solution, 4.05g of $FeCl_3$ was dissolved in 500ml of distilled water.
  - Solution (b): To prepare 10% solution, 8.11g of $FeCl_3$ was dissolved in 500ml of distilled water.

- **Lead acetate:** A 10% basic lead acetate solution is used for the detection of flavonoid. To prepare, 5g of lead acetate was dissolved in 500ml of distilled water.

Composition of Nutrient Broth Media

Ingredients	**Gms/ Liter**
Beef Extract	3.0
Peptone	5.0

Composition of Muller Hinton Agar Media

Ingredients	**Gms/Liter**
Beef extract	2.0
Acid hydrolysate of casein	17.5
Starch	1.5
Agar	17.0

Composition of Sabouraud Dextrose Agar Media

Ingredients	**Gms/Liter**

Dextrose	40.0
Peptone	10.0
Agar	15.0

ABSTRACT

Name: Saumya Jaiswal

Id. No: 4890

Degree: M.Sc. Biotechnology

Department: Department of Cell Biology

Thesis title: ***"A study on phytochemical profiling and antimicrobial activity of Cymbopogon citratus (Lemon grass)"***

In recent years researchers have been paid attention to find out new alternative sources of antimicrobial agents especially from plant sources. *Cymbopogon citratus* (Lemon grass) is an aromatic medicinal plant in the family Poaceae. The main chemical components of lemon grass are: myrcene, geranial, limonene, citronellol, neral, caffeic acid, citral have been used extensively for many years in soap, perfumery, and detergents and pharmaceuticals. Phytochemical screening of the plant leaf reveals that the presence of saponins, alkaloids, flavonoids, terpenoids, phenols, tannins and glycosides. Different leaf extracts of *Cymbopogon citratus* leaves were prepared and its antimicrobial activity were evaluated by agar well diffusion method against both Bacterial species such as: *Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus epidermidis*, *Escherichia coli* as well as Fungi pathogens such as: *Aspergillus fumigatus*, *Candida albicans*, *Penicillium Chrysogenum*. The antimicrobial activity of crude methanolic, ethanolic, acetone and aqueous extract of *C. citratus* was evaluated to find the zone of inhibition, so that Antimicrobial activity of various extract of leaves of *Cymbopogon citratus* was carried in attempt to develop a new pharmaceutical drug from natural origin for prevention of pathogenic microbes.

Key words: *Cymbopogon citratus*, Lemon grass, Phytochemicals, Zone of Inhibition, Extract, Microbes

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Id.No.4890 Advisor

Curriculum Vitae

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CAREER OBJECTIVE:

Seeking a position to utilize my skills and abilities and use them in an esteemed organization that enjoys an excellent reputation for career and personal development and to deliver the standards of service. Also looking forward to use my knowledge in some active implementation.

QUALIFICATION:

Exam	Year	College & Board	Main Subjects
M.Sc. Biotech	2019-2021	Sardar Vallabh Bhai Patel University of Agriculture and Technology, Meerut.	Biotechnology
B.Sc.	2015-2018	Sam Higginbottom University of Agriculture, Technology And Sciences, Prayagraj.	Biotechnology
12^{th}	2013	Dr. Rizvi Springfield School, Karari Kaushambi	PCB, English, Hindi
10^{th}	2011	Dr. Rizvi Springfield School, Karari Kaushambi	English, Maths, Science, Hindi, Social studies

TRAINING:

Organization	Training Topic/Assignment	Duration	Remarks
Helix Biogenesis, Noida	Basic Techniques of Molecular Biology.	15 days	Completed successfully
CSIR-Institute of Himalayan Bioresource Technology	Extraction, purification, identification & estimation of polyphenols from tea-shoots.	1 month	Completed successfully
S.G.R.R.(P.G.) College Dehradun, Uttarakhand	Mushroom Cultivation.	1 month	Completed successfully

INSTRUMENT OPERATION & CALIBRATION KNOWLEDGE:

- UV-Spectrophotometer
- Hot Air Oven
- Electronic Weighing Balance
- Water Bath
- Laminar Air Flow

KEY STRENGTHS:

- Believe in team Spirit and hard work.
- Positive Attitude.
- Optimistic and self-motivated.

HOBBIES:

- Listening music.
- Playing games.
- Watching movies.

PERSONAL DETAILS:

- Father's name : Mr. Shiv Chandra Jaiswal
- Date of Birth : 25/10/1996
- Sex : Female
- Marital Status : Unmarried
- Nationality : Indian
- Language Known : English, Hindi

Permanent Address : Janghai Bazar Prayagraj, U.P, 212401