Bioprospecting of Medicinal Plants from Goa for their Antibacterial and Quorum Quenching Activity

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DECLARATION BY STUDENT

I hereby declare that the data presented in this dissertation entitled "Bioprospecting of Medicinal Plants from Goa for their Antibacterial and Quorum Quenching Activity" is based on the results of investigations carried out by me in the Microbiology programme at the School of Biological Science and Biotechnology, Goa University under the supervision of Dr. Milind M. Naik, Assistant Professor in Microbiology, Goa University and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/experimental or other findings given in the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report entitled "**Bioprospecting of Medicinal Plants from Goa for their Antibacterial and Quorum Quenching Activity**" is a bonafide work carried out by **Ms. Priyanka Umanath Prabhu** under my supervision in partial fulfillment of the requirements for the award of the degree of **Master of Science in Microbiology**, in the Microbiology programme at the School of Biological Sciences and Biotechnology, Goa University.

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CONTENTS

PREFACE

The emergence of pathogens resistant to antibiotics has become a major health concern, creating a threat to the prevention and management of a wider range of diseases brought on by bacteria, viruses, parasites, and fungi. 70% of the bacterial infections are Multi Drug Resistant (MDR) and this affect country's economy and health. A low-cost and reliable substitute is urgently needed since antibiotics are expensive and seriously affect middle-class and lower-class families. The greatest supply of chemical compounds is found in medicinal plants, whose use has grown significantly because of their low toxicity, antibacterial, and antioxidant properties. Promising antibacterial action against bacterial pathogens is revealed by organic solvent extracts of medicinal plants' leaves and bark. As a result, a low-cost and dependable substitute for treating multidrug resistant illnesses is need of an hour. Therefore, bioprospecting of medicinal plants from Goa for their antibacterial and quorum quenching properties to develop novel medicines with higher antimicrobial properties can be of great significance.

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TABLES

FIGURES

ABBREVIATIONS USED

Entity	Abbreviation
Acylated Homoserine Lactone	AHL
Adenosine Triphosphate	ATP
American Type Culture Collection	ATCC
Deoxyribonucleic acid	DNA
Ethanolic Extract	EE
Ethyl Acetate	EA
Methanolic Extract	ME
Millimolar	mm
Multi Drug Resistance	MDR
Multi Drug Resistance Organisms	MDRO'S
Quorum Quenching	QQ
Quorum Sensing	QS
Sodium hydroxide	NaOH

Abstract

The multi drug resistance (MDR) infections are one of the major health concerns in the modern era. Therefore, discovering new natural remedies to treat MDR infections is need of an hour. Medicinal plants have been used in traditional medicine for centuries. They offer a natural alternative to synthetic drugs and can have various therapeutic properties. The present study describes the antibacterial, quorum quenching activity and phytochemical index of seven different medicinal plants (*A. paniculate, A, parviflora, C. melo, C. amboinicus, S. trilobata, P. cineraria, C. ternatia*). For present investigation extracts of these plants were obtained by maceration method in methanol, ethanol, ethyl acetate and n-hexane solvents and were compared for their antibacterial and quorum quenching activity using Agar well diffusion method. Compared to other solvent extracts of these plant methanolic extract showed better antibacterial and QQ activity against all the tested pathogenic strains. Out of the 7 plants, the *P. cineraria* plant sowed highest antibacterial and quorum quenching potential.

Our findings provided evidence that organic solvent extracts of these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of different diseases.

Keywords: Medicinal plants, Antibacterial activity, Quorum quenching activity, phytochemicals.

1.
INTRODUCTION

1.1 Background

Bioprospecting refers to exploring and discovering new sources of chemical compounds and other valuable products from nature. Nature is indeed a rich source of medicinal agents (Shresha et.al 2007). Products from nature had been the basis for developing numerous drugs, including antibiotics, painkillers, and anticancer agents. For thousands of years, 80% of the worldwide population depend on herbal medicines for various ailments. Many plants, fungi, microorganisms produce compounds with therapeutic properties (Alam et.al 2009).

Medicinal plants are considered as the most useful plants as they exert beneficial effect on the human or animal body. The extracts from different parts of plants have been developed to use as an antimicrobial substance (campo et.al 2000). Certain organic components found in medicinal plants have specific physiological effects on humans. These compounds are known as bioactive substances and include terpenoids, tannins, carbohydrates, steroids, flavonoids and alkaloids. Living organisms produce these substances through their primary or more accurately secondary metabolism. Secondary metabolites are incredibly diverse substances with unknown functions in terms of chemistry and taxonomy. They are extensively employed in numerous fields, including scientific research, veterinary medicine, agriculture, and human therapy. It has been demonstrated that a wide variety of phytochemicals from various chemical classes have inhibitory effects on microbes of all kinds in vitro. The combination of phytochemicals varies from one plant species to another. These phytochemicals don't have any negative effects, in contrast to pharmaceutical compounds. Phytochemicals are therefore referred to as "man-friendly medicines" because they treat illnesses without harming people.

The need for more and more medications obtained from plants is growing all the time. For this reason, it is crucial to systematic evaluate medicinal plants for a range of conditions that are treated in traditional medicine. Certain medicinal plants' leaves and bark were extracted using organic solvents, and the results showed good antibacterial efficacy against the Gram-

positive and Gram-negative strains of bacteria (Panda et al., 2009). Previous studies have discovered the antibacterial properties of many plant extracts using different organic solvents, like methanol, petroleum ether, acetone-hexane and ethanol. Medicinal plant studies uses dried and fresh both samples, with dried being preferred due to experimental design time. Decreasing particle size increases surface contact with extraction solvents, while powdered samples have a more homogenized, smaller particle size for efficient extraction. According to Methods Optimization in Accelerated Solvent Extraction, effective extraction necessitates solvent interaction with target analytes and particle size lower than 0.5 mm. Preparing plant samples to preserve the biomolecules in the plants prior to extraction is the first step in the study of medicinal plants. Plant material, either fresh or dried, can yield plant samples such as Flowers, fruits, barks, leaves and roots; further plant material preparations such as grinding and drying can affect how long phytochemicals remain in the final extracts. (Mukherjee et al. 2011) The process of extracting medicinally active compounds from plant (and animal) tissues involves conventional processes and the use of specific solvents. These extraction methods remove the insoluble cellular marc from the soluble plant metabolites. The resulting plant products are mostly complex combinations of metabolites that can be used orally or topically. They can be found in liquid, semisolid, or dry powder form. Standardized extraction techniques are used to obtain the therapeutically desirable components of crude pharmaceuticals, or medicinal plant parts, and to remove undesired material by treating them with a selective solvent.

1.1.1 Emergence of Multi Drug Resistance (MDR) microbes in community

The increasing global issues regarding the Multi Drug Resistance (MDR) increased the interest of determining the antibacterial activities of different compounds. Due to easy excess and constant exposure to commercially available drugs it is assumed that most of the

pathogenic organisms had gain resistance towards them. Therefore, it is a need of an hour to determine different compounds that can be used to made medicine with higher antimicrobial properties (Archana et al. 2011).

Drug and antibiotic-resistant bacteria are the most common cause of illnesses nowadays, and some pathogens have even developed resistance to a wide range of antibiotic classes. Such organisms are called Multidrug resistant organisms (MDROs). The genes of an antibiotic resistance are present on the transposons, integrons and plasmid. The resistance can be achieved in bacteria either by intrinsic or extrinsic mechanisms. Mechanisms those are stated by naturally occurring genes found on the hosts chromosome are intrinsic mechanisms. Mechanisms those involve mutations by the antibiotic used in the target genes are the extrinsic mechanisms and the transfer of resistance take place by plasmids, bacteriophage, transposons, and other mobile genetic material (Alekshun et al. 2007).

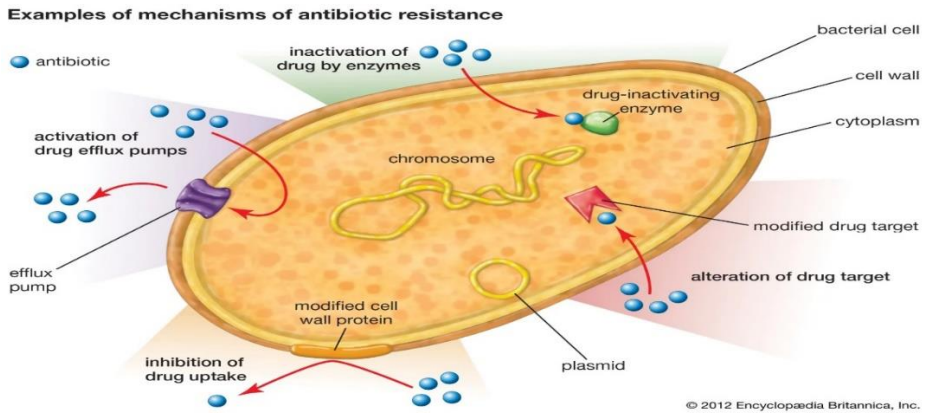


Fig 1.1 .1a: Mechanisms of antibiotic resistance (Bbosa et al., 2014).

1. Efflux pump: The efflux pump causes bacteria to come resistant to antibiotics in the first place (Regli etal.2006). According to Webber et al (2003), an efflux pump is the transport protein that pushes antibiotics or other dangerous accoutrements out of the cell and into the girding environment. Tetracycline was the first antibiotic to have its efflux pump medium

illustrated(Mcmurry et al. 1980). The resistance- nodulation- cell division (RND), major facilitator (MF), staphylococcal/ small multidrug resistance (SMR), ATP- list mail (ABC), and multidrug and poisonous emulsion extrusion (MATE) families are the five protein families of efflux proteins involved in medicine resistance. Primary or secondary transport might be the source of efflux. ATP hydrolysis drives primary transport, secondary transport is driven by secondary transport, and proteins in ABC are driven by primary transport.

2. Chromosomal mutation: If a mutation occurs in the chromosomal gene that the treatment is targeting, the organism may develop antibiotic resistance. Mutations in DNA gyrase and topoisomerase, two of the medicine's targets, can be used, for case, to identify fluoroquinolone resistance.

3. Genomic duplication: Antibiotic resistance in eukaryotic cells is achieved through gene modification. Multi-drug transporters and medicine targets are overexpressed (Albertson, 2006; Alekshun et al. 2007).

4. Regulatory gene: In bacteria, MDR is regulated by gene- decoded nonsupervisory proteins that serve as activators or impediments of recap or restatement of specific antibiotic target genes (Schumacher and Brennan, 2002). In *E. Coli*, Mar A protein regulates the expression of MDR efflux pump and other proteins involved in antibiotic vulnerability (Barbosa and Levy, 2000).

5. Horizontal gene transfer: Another system by which organisms acquire antibiotic resistance is horizontal gene transfer. It's the process by which inheritable material, or DNA, is transferred from one species of bacteria to another, whether they're affiliated or not. This can be fulfilled through three different mechanisms conjugation, metamorphosis, and transduction. In transduction two nearly spaced bacterial cells change inheritable material using bacteriophage, a type of bacteria-specific contagion. DNA is released into the external

development during cell lysis or cell death. Such a little scrap of DNA is incorporated into the cell by the process known as metamorphosis, and when inheritable material is transferred between cells directly, the process occurs is called conjugation (Todar 2008).

1.1.2 Quorum Sensing (QS)

The communication mechanism that bacteria uses to coordinate their actions Is called Quorum sensing. Autoinducers are tiny signaling molecules that they release and detect. These molecules have a threshold concentration at which they start to express particular genes, which can result in collective behaviors like virulence or the creation of biofilms.

Bacteria can engage in a variety of collective behaviors in response to quorum sensing, including the production of biofilms, which are protective layers formed by clumps of bacteria. Furthermore, it controls the synthesis of virulence factors, which permits bacteria to plan coordinated assaults on their hosts. Other instances include the discharge of toxins, the release of enzymes, and the synchronization of bioluminescence in some bacterial species.

Bacteria classified as Gram-positive or Gram-negative use quorum sensing. Acylated homoserine lactone (AHL), which is produced by LuxI type of enzyme—a signal synthase expressed by the first gene of the Lux operon—is the autoinducer produced by gram-negative bacteria. More than 25 species of Gram-negative bacteria have been shown to have quorum sensing circuits. A Gram-negative bacterium such as *Chromobacterium violaceum*, which can be found in soil and water and produce various substances like proteases, cyanide hydrogen, antibiotics, and chitinase. Of particular interest is a water-insoluble purple pigment that is involved in the phenotypic response to AHLs. Peptides synthesized from precursors for quorum sensing are used as autoinducers by gram-positive bacteria. Furthermore, oligopeptide signaling systems are displayed by a wide variety of Gram-positive bacteria in

addition to AI-2. Bacteria are able to detect the population density of other organisms in their vicinity by developing and reacting to a combination of these signals (Nazzaro et al., 2013).

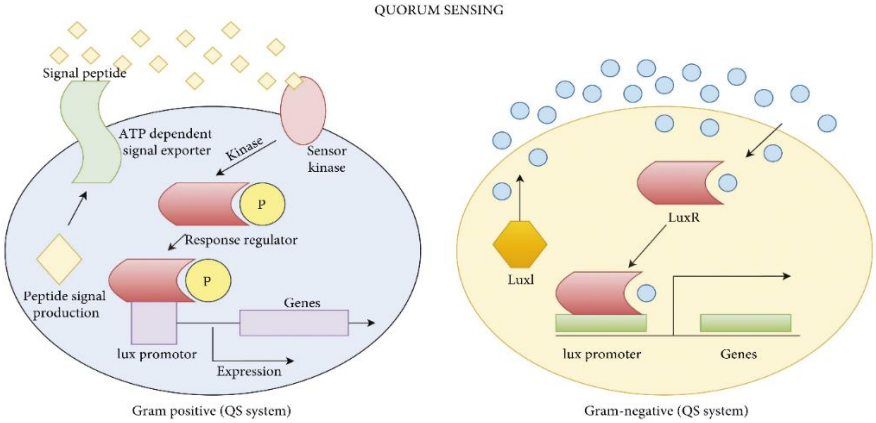


Figure 1.1.2a: Quorum sensing arrangement in Gram-positive and Gram-negative bacteria (Subramanian et al. 2022)

1.1.3 Quorum Quenching (QQ) mechanisms

The expression of bacterial QS can be silenced or inhibited through various means, including: (i) blocking AI-2 synthase to inhibit AI biosynthesis in Gram-negative bacteria; (ii) QS signal degradation in the extracellular environment by Quorum-Quenching (QQ) enzymes like AHL-lactonase, oxidoreductase, and acylases; (iii) blocking receptors or interfering with the AI/receptor complex; (iv) attenuation of the QS signal as a result of a complex formation between AI and molecular imprinting polymers (IMPs); or (v) degradation of enzymes that interfere with cell-cell communication, leading to the active control of the AI-2 concentration or availability (Ravichandiran et al. 2013; Machado et al. 2020).

Inactivation or complete degradation of the generated signal molecules can be achieved by different methods: chemical degradation, enzymic destruction and metabolism of the AHL.A simple way to achieve inactivation of the AHL signal molecules is by increasing the pH to above 7; this causes lactonolysis – ring opening – of the AHL (Yates et al., 2002). Enzymic activity can also be used to lactonolyze AHLs. The activity of these enzymes lowers the amount of bioactive AHL signal molecules by catalyzing the ring-opening reaction. Within 2

h, up to 20 mM 3-oxo-C6 HSL can be completely in-activated by a suspension culture producing the enzyme. It seems likely that production of AHL-degrading enzymes constitutes a non-antibiotic-based strategy employed by some bacteria in the competition with AHL producers. Enzyme-based method to inactivate the signal molecules is simply to metabolize the AHLs. Both *Variovorax paradoxus* and *P. aeruginosa* PAI-A are able to proliferate with AHLs as sole source of energy, carbon and nitrogen. The bacteria produce an amino acylase which cleaves the peptide bond of the signal molecule. The sidechain is used as carbon source, the nitrogen from the amide bond is made available as ammonium via the action of lactonases and the ring part is used as energy donor (Huanget al., 2003; Leadbetter & Greenberg, 2000).

Interference with the signal receptor: One widely explored method is to block the receptor with an analogue of the AHL signal molecule. There are basically three ways to develop on the AHL scaffold: introduction of substitutions in the acyl sidechain which at the same time maintain the lactone ring, introduction of substitutions and alterations in the lac-tone ring which at the same time leave the acyl side chain unchanged, and finally extensive modifications in both the acyl side chain and the lactone ring. Agonistic AHL analogues carried an acyclic or cyclic alkyl substituent on the outmost carbon atom of the side chain. However, by replacing the C-3 atom with sulphur in the acyl side chain, Persson et al. (2005) created analogues able to block expression in both LuxR- and LasR-controlled QS reporters.

By stopping the AHL molecule from attaching to its receptor, quorum sensing can be suppressed. It may be the result of chemicals binding to the receptor more preferentially than the AHL molecule, causing competitive inhibition.

1.1.4 Quorum Quenching (QQ)

Quorum quenching is a phenomenon where bacteria produce enzymes or molecules to interfere with quorum sensing, the mechanism by which bacteria communicate and coordinate gene expression based on population density. In quorum sensing, bacteria use different types of molecules as autoinducers to communicate with each other. The specific type of autoinducer depends on the bacterial species. Some bacteria use acyl-homoserine lactone (AHL) molecules as their autoinducers, while others use peptides or other small molecules. Each type of autoinducer possess unique structure and chemical properties, allowing bacteria to recognize and respond to them ((Parsek et al., 1999).

Chromobacterium violaceum and Serratia marcescens are both known to exhibit quorum quenching mechanisms. In *C. violaceum*, the quorum sensing system works through the production and detection of signaling molecules called autoinducers. When the bacteria reach a certain population density, they release these molecules into their environment. As the concentration of the molecules increases, they bind to specific receptors on the bacterial cells, triggering a series of gene expression changes. These changes can lead to the production of various compounds, including the purple pigment violacein, which gives the bacteria its distinctive color. In *C. violaceum*, it produces a pigment called violacein, if any QQ molecule interfere with quorum sensing system which was required for pigment production than pigment will not produce thereby disrupting their communication and potentially their ability to form biofilms or express virulence factors. Similarly, *Sarratia marcescens* prodigiosin pigment is controlled by QS. If QQ molecule inhibit or degrades AHL then prodigiosin pigment will not produce. That’s why these bacteria were used as bioreporters. Some pathogens produce valencenes factor like biofilm production, enzymes, hemolysin etc. If we want to cure diseases caused by them, we need to search for QQ molecules which will inhibit QQ molecules.

1.1.5 Impact of Phytochemicals on Quorum Sensing

The phytochemicals quercetin, myricetin, kaempferol, baicalin, eugenol, and 6-gingerol found in plant extracts operate as quorum sensing inhibitors.

Medicinally bioactive compounds derived from plant extracts that exhibit anti-quorum sensing activities are known as Quorum quenchers. Among the major quorum sensing inhibitors are flavanones, which include naringenin, eriodyctiol, and taxifolin (Vandeputte et al., 2011), and flavonoids, which include quercetin, myricetin, kaempferol, and eugenol (Limos et al., 2018). Numerous phytochemical extracts block quorum sensing by preventing AHL from acting because of their structural similarities and by speeding up the degradation of AHL inhibitors' LuxR/LasR receptors. Fruit, herb, and spice extracts were found to have superior quorum sensing inhibitory action at sublethal dosages. It has also been shown that phytochemicals can limit AHL activity by controlling the pathogens' synthesis of AHL through the combination of two distinct mechanisms (Gemenez et al., 2012).

Cumarin, a phenolic molecule found in cinnamon, have QQ activity. The bark of Dalbergia trichocarpa contains a new bioactive chemical called oleanolic aldehyde coumarate (OLAC), which is an excellent quorum sensing inhibitor. 6-gingerol is a smelly oil obtained from fresh ginger and rosmarinic acid (Kim et al., 2015, Corral et al., 2016). When flavonoids are given to a pathogen, their ability to produce virulence factors is suppressed. The phytochemical extracts from certain natural sources interfered with AHL's activities. Many spices and herbs demonstrated a positive response against biofilm formation by bacterial pathogens and violacein pigment production in *Chromobacterium violeceum.*

1.2 Aims and objectives

Aim: To study Antibacterial and Quorum Quenching potential (QQ) of different solvent extracts of selected medicinal plants.

Objectives:

1. Selection of medicinal plants for their Antimicrobial and QQ potential
2. Preparation of plant extracts of Medicinal plants in different solvents.
3. Screening of plant extracts of medicinal plants for antimicrobial and quorum quenching potential.
4. Qualitative determinations of phytochemical constituents in medicinal plant extracts.

1.3 Hypothesis

The negative impact of synthetic substances on health is the major concern in the modern era. Plants are the richest source of diverse chemicals, which are used not only in traditional system of medicines but also as a source of lead molecules for synthetic drugs. The most prominent of these bioactive compounds are alkaloids, phenols, flavonoids and tannins. Organic solvent extracts of leaves and bark of medicinal plants revealed the promising antibacterial activity on bacterial pathogens. Therefore, bioprospecting of medicinal plants from Goa for their antibacterial and quorum quenching potential can be a cheap and reliable alternative to treat MDR infections.

1.4 Scope

Studying the antibacterial and QQ potential of medicinal plants could help us discover new natural remedies to treat MDR infections. Quorum sensing based technologies can aid in the bioremediation of polluted environments by promoting breakdown of harmful substances, which can lead to infections

2.
Literature Review

2.1 Medicinal plants extract as novel antimicrobial compound

Medicinal plants are considered precious in both Chinese traditional medicine and Ayurveda and are a major part of the many traditional medicinal practices used in the Himalayan region (Ghimire et al., 2005). Numerous chemical molecules are produced by plants and are categorized into primary and secondary metabolites according to their functional groups, chemical class, and place of biosynthesis. Primary metabolites are directly involved in growth and development. Secondary metabolites can act as biocatalyst but are not directly involved in growth of plants. Plants biosynthesize secondary metabolites for a variety of functions, including as defense against diseases and predators, inter- and intra-specific interactions, and growth regulation (Verpoorte et al. 2000).

A variety of secondary metabolites are identified in medicinal plant extracts, and they are employed as chemotherapeutic agents or as building blocks for the creation of contemporary medications.

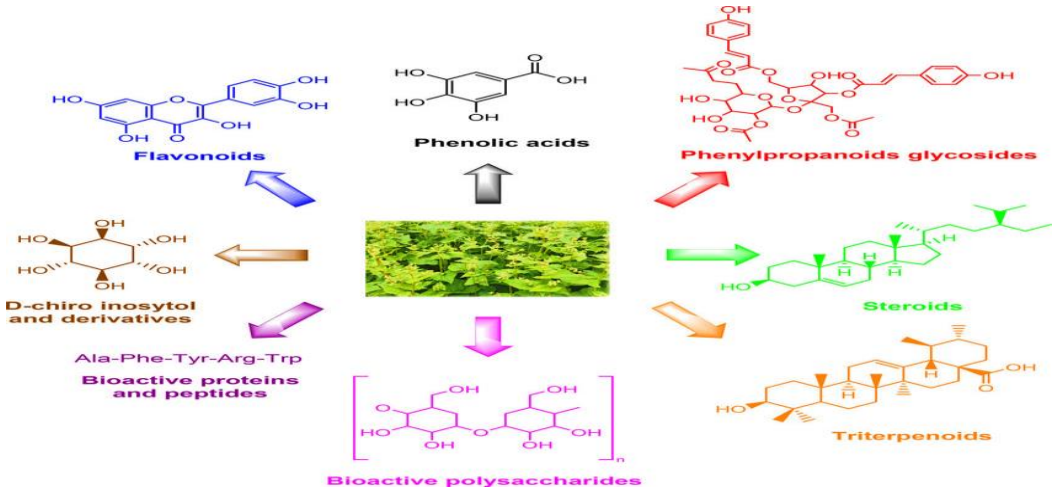


Figure 2.1.1 Different phytochemicals present in medicinal plants (Zou et al.2021)

It has been noted that saponins show hemolytic exertion, cholesterol list rates, rush and coagulation of red blood cells, antifungal exertion, and an inhibitory effect on inflammation (Sodipo et al. 1991). Since ancient times, alkaloids have been used medicinally due to its

natural characteristics, including cytotoxicity, analgesic, antispasmodic and antibacterial, and antiviral (Stray et al. 1998). multitudinous publications state that glycosides are known to reduce blood pressure. Tannins and terpenoids are allowed to have analgesic and anti-inflammatory parcels. In addition, tannins have the capability to be tangy and have antibacterial parcels, which speed up the mending of injuries and seditious mucous membranes. Phenolic motes are among the most significant and most probably the largest class of secondary metabolites. They've a wide range of natural characteristics, including the capability to help cardiovascular complaint, decelerate down angiogenesis and cell division, inhibit angiogenesis, ageing, reduce inflammation, help atherosclerosis, and cover the heart (Han et al. 2010). Phenolic compounds are mostly reported in the plant extracts that have high antioxidant rates. Flavonoids, phenolic acids, tocopherols, and other phenolic composites set up in shops are the primary source of natural antioxidants. The flavonoids are the most abundant and well- delved natural phenols. They've potent anticancer parcels and are also largely important antioxidants (Salah et al. 1998).

1.***Andrographis paniculata*** **(Kiraytem)**

Fig. 2.1a: *A. paniculate* plant

Scientific classification

Kingdom- Plantae

Order- Lamiales

Family- Acanthaceae

Genus- Andrographis

Species- *A. paniculate*

For ages, the Acanthaceae (Acanthus) family member *A. paniculata* has been utilized as a medicinal herb to treat fever, herpes, upper respiratory and gastrointestinal tract infections, and other chronic illnesses. Its pharmacological effects are diverse. Diterpene lactone the main therapeutic element of Andrographolide is found in *A. paniculata*. The andrographolide that present in this plant possesses anti-cancer properties (Sheeja and Kuttan 2007). According to Siripong et al. (1992), the other active ingredients are streptasterol, homoandrographolide, 12-dihydroandro grapholide (andrographolide D), 14-deoxy-11, and andrographosterin. In their research, Zaidan et al. (2005) used the disc diffusion method to

screen five indigenous medicinal plants for antibacterial activity in vitro. They discovered that aqueous extract of A. paniculate has antibacterial activity against both gram-positive and gram-negative bacteria.

According to Najib et al. (1999), the extracts of *A. paniculata* prepared in chloroform are effective against the malarial parasite. The five gram-negative bacteria that were employed as pathogenic strains to investigate the antibacterial activity of *A. paniculata* were *Klebsiella pneumoniae, Salmonella typhimurium, Enterobacter cloacae, E. coli, Pseudomonas aeruginosa,* and four gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis, and Enterobacter faecalis*). Agar well diffusion method was used to determined antibacterial activity of *A. paniculate* (Okeke et al., 2001). Till date there is no report on Kirayte for Quorum quenching potential.

2.Artemesia parviflora (Maanpatri)

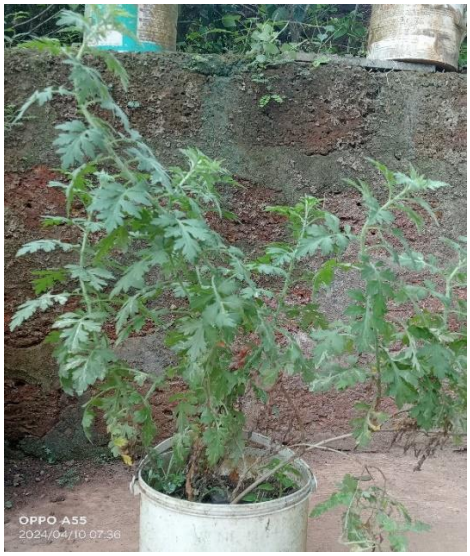


Fig 2.1b: *A. parviflora* plant

Scientific classification

Kingdom: - Plantae

Order: - Asterales

Family: - Asteraceae

Genus: - Artemesia

Species: - A. *parviflora*

Essential oils from the Asteraceae family plant *Artemisia* are well-known. *Artemisia*'s powerful scent and bitter flavor is because of the Terpenoids and sesquiterpene lactones present in their essential oil (Tomlinson et al. 2010). *Artemisias* essential oils have been used for many years in medicine for a range of ailments, including nematocidal, fungicidal, antibacterial, and antiviral (Meneses et al. 2009). Antimicrobial activity of *Artemisia* spp. against multidrug resistant strains of opportunistic pathogens widely distributed in hospitals and increasingly isolated from community acquired infections, such as *K. pneumoniae, P.*

aeruginosa, E. coli, Bacillus subtilis, and Staphylococcus aureus, had been reported (Ramezani et al. 2004).

According to reports, artemisia species have significant concentrations of flavonoids and phenolic compounds, which possesses high antioxidant and radical-scavenging abilities (Shi et al. 2010). Using the agar dilution method, the Antibacterial Activity (AA) of the *A. parviflora* leaf extracts was determined against ten therapeutically relevant pathogens. For the majority of the test organisms, *A. parviflora* extracts showed significantly wider inhibitory efficacy. Till date there is no report on *A. parviflora* for Quorum quenching potential.

3. Clitorea ternatea (Aparajita)

Fig 2.1c: *C. ternatia* plant

Scientific Classification

Kingdom: - Plantae

Order: - Fabels

Family: - Fabaceae

Genus: - Clitoria

Species: - C. *ternatea*

Clitoria ternatea L., sometimes known as Butterfly Pea, is a perennial twining herb that grows in tropical equatorial regions. It is used in natural food coloring as a traditional medicinal herb (Anonymous 1998). Many ailments have been treated using C. *ternatea* L. variants, a member of the fabaceae family, in traditional medicine and ayurveda. Research has demonstrated that *C. ternatea* possesses a variety of pharmacological properties, including anxiolytic, antidepressant, and antistress properties. Antimicrobial, analgesic, sedative, antipyretic, and anti-inflammatory properties (Gomez and Kalamani, 2003).

There have been reports of tranquilizing, anti-inflammatory, and anti-pyretic properties in various portions of *C. ternatea.* According to reports, the flavanol glycoside found in roots has anti-bacterial properties, and *Clitoria* has anti-inflammatory, hepatoprotective, anti-hyperlipidemic, and immune-inhibitory properties (Mukherjee et al., 2008). Phytochemical components with significant pharmacological activity are present in C. *ternatea* variants. C. *ternatea* has been found to have a broad variety of bioactive molecules, such as triterpenoids, flavanol, glycosides, anthocyanins, and steroids. Its extracts also have a broad variety of therapeutic properties, such as antibacterial, antipyretic, and anti-inflammatory effects. The Antibacterial Activity (AA) of C. *ternatea* L. leaves and roots against *E. coli* was proven. Use of methanolic extracts increased the potency of C. *ternatea* leaves' inhibitory zones in the Disc Diffusion method (Anand et al., 2011). But till date, no report on QQ potential of *C. ternatia* was reported.

It has been observed that *C. ternatea* contains significant phytoconstituents in total phenols, which are quite greater than tannins and are followed by flavonoids.

4. Cucumis melo var. agrestis (Karit)

Fig 2.1d: *C. melo* L. plant

Scientific Classification

Kingdom: - Plantae

Order: - Cucurbitales

Family: - Cucurbitaceae

Genus: - Cucumis

Species: - Cucumis melo var. agrestis

Numerous medical benefits associated with *C. melo* include its ability to reduce pain, fight inflammation, scavenge free radicals, diuretic, anti-microbial, anti-helminthic, anti-diabetic, anti-cancer, and anti-ulcer effects. According to Milind et al. (2011), the phytoconstituents of *C. melo* that give it its unique therapeutic qualities include amino acids, carbohydrates, fatty acids, glycolipids, phospholipids, β-carotenes, flavonoids, terpenoids, chromone derivatives, ascorbic acid, volatile components, and different minerals.

Cucumis melo L. demonstrated Antibacterial Activity (AA) in vitro against *S. aureus, E. coli P. aeruginosa, P. mirabilis*, and *E. faecalis* frequent urinary tract pathogens. In literature there is no report on *C. melo* for its QQ potential.

5. Sphegneticola trilobata (Yellow creeping daisy)

Fig 2.1e: *S. trilobata* plant

Scientific Classification

Kingdom: - Plantae

Order: - Asterales

Family: - Asteraceae

Genus: - Sphegneticola

Species: - *S. trilobata*

Asteraceae family member *S. trilobata* has been used in ancient Chinese, Indian, and Caribbean medicine as well as in Central and South American traditional medicine (Mishra et al 2011). According to Marina et al. (2020), *S. trilobata* (L.) is a medicinal plant with benefits for treating ulcers, sore throats, varicose veins, headaches, fever, epilepsy, amenorrhea, snakebite, wounds, renal dysfunction, hepatitis, colds, and dyspepsia. The plant has been

shown to exhibit bioactivities that include anticancer, hepatoprotective, antifungal, antioxidant, antibacterial, and anti-inflames (Halimatussakdiah et al., 2020).

78.80% of MCF-7 cancer cells (Breast cancer) were subjected to apoptosis when exposed to an EA (ethyl acetate) extract derived from *S. trilobata* leaves (Mardina et al., 2020). Additionally, it possesses comparable efficacious inhibitory activities to commercial medications against Gram-negative *E. coli* and *S. typhi*. The methanolic extract's bioactivate properties are due to the presence of flavonoids, alkaloids, phenols, saponin, and tannin. The primary chemical components of *S. trilobata*, including luteolin, flavonoids, tannins, essential oils and kaurenoic acid were identified from the aerial sections of the species (Filho, 2000; Fedelis et al., 2005; Silva et al., 2012). According to Leite et al. (2019), a crude hydroalcoholic extract derived from the leaves of *S. trilobata*, which is rich in flavonoids and terpenes as secondary metabolites, has antibacterial action against bacterial cultures isolated from human and dog skin. But till date there is no report on *S. trilobata* for its QQ potential.

6.Plectranthus amboinicus (Vatelav)

Fig 2.1f*: P. amboinicus* plant

Scientific Classification

Kingdom: - Plantae

Order: - Lamiels

Family: - Lamiaceae

Genus: - Plectranthus

Species-: *P. amboinicus*

The well-known plant *Plectranthus amboinicus* is a part of the Lamiaceae family. This plant has been used to make traditional remedies because of its therapeutic properties, such as syrups. In addition, it can be also used to treat bronchitis, epilepsy, and other illnesses. It contains flavonoids including apigenin, luteolin, and salvigenin, according to a photochemical analysis (Janakiraman et al., 2014). Famous illnesses including cephalgia, otalgia, anorexia, dyspepsia, bloating, colic, diarrhea, cholera, gums, seizures, asthma, cough, chronic bronchitis, and renal calculi are all cured with this plant extracts (Aragao et al.,2006).

The antibacterial properties of *P. amboinicus* leaf essential oil was examined by Agar well diffusion method. According to the reports, *P. amboinicus* (Lour.) essential oil possesses more antibacterial action against *Staphylococcus aureus* (Gram-positive) bacteria than against *Escherichia coli* (Gram-negative). According to Hassani et al. (2012), the minimum concentration of inhibition for *E. coli* and *S. aureus* was 0.2% and 0.1%, respectively. According to Koti et al. (2011), *P. amboinicus* ethanol extracts have antibacterial activity at a concentration of 50 g/ml against *Streptococcus mutans.*

Due to its ability to stop in vitro denaturation, plant extract exhibits anti-inflammatory properties. The existence of polyphenolic content causes effects, which results from many compounds working together synergistically (Rao et al., 2017). There is no report on QQ potential of *P. amboinicus*.

7.*Prosopis cineraria* (Shami plant)

Fig 2.1g: *P cineraria* plant

Scientific Classification

Kingdom: - Plantae

Order: - Fabels

Family: - Fabaceae

Genus-: Prosospis

Species: - *P. cineraria*

Prosopis cineraria, sometimes referred to as Jand in the area, is a part of the second-largest family of plant that produces flowers, the Leguminosae (Khatri et al. 2010). Additionally, *prosopis* spices have been employed as a folk remedy for a variety of illnesses in indigenous medical systems. The flowers are given to the patient along with sugar in order to stop miscarriage (Gupta et al., 2014).

Gram-negative and gram-positive organisms were also susceptible to the effects of the extracts of plants. Aqueous extract had a very mild effect on *S. aureus, S. pneumoniae,* and *E. coli*, while methanol extract was shown to be more effective against all microorganisms, exhibiting 11 mm and 10 mm zones of inhibition, respectively. While methanol extract has a higher concentration of phytochemicals like glycosides, alkaloids and volatile oils—all of which are present in *Prosopis cineraria* in greater abundance—aqueous leaf extract showed

less antibacterial activity than methanol extract. flavonoids, tannins, steroids, saponins, alkaloids, and glycosides are all present in *P. cineraria*.

According to Singh et al. (1998*), P. cineraria* is widely used to cure boils, skin conditions, and as a blood purifier. Best Antibacterial Activity (AA) was shown by leaf extract against every strain of bacterium and fungus. Because of their high phenolic content, the extracts also showed notable antioxidant activity. The production of antioxidant and antibacterial compounds may benefit from the utilization of the plants (Mohan et al., 2017). Till date there is no report on Quorum quenching potential of *P. cineraria* plant.

3.
Materials and Methods

3.1 Plant material

The medicinal plant used in this study consisted of leaves and fruit of following medicinal plants. These Medicinal plants were selected because they are native to Goa and are used in traditional medicinal preparations since ancient time

Common name	Botanical name
Manpatri	*Artemesia parviflora*
Kiraytem	*Andrographis paniculata*
Shami plant	*Prosopis cineraria*
Yellow creeping daisy	*Sphagneticola trilobata*
Aprajita	*Clitoria ternatea*
Vatelav	*Coleus amboinicus*
Karit	*Cucumis melo L.*

Table1: Plant Used to study Antibacterial and Quorum Quenching potential

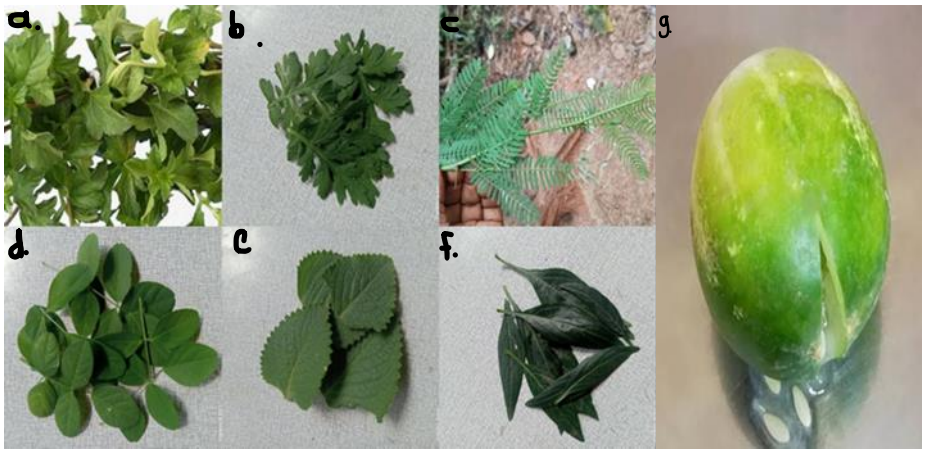


Figure 3.1.1 Collected leaves for extraction of a) *S. trilobata* b) *A. parviflora* c) *P. cineraria* d) *C. ternatea* e) *C. amboinicus* f) *A. paniculate* and fruit of g) *C. melo L.*

3.2 Preparation of plant extracts

Fresh plants were gathered and given separate washings in d/w (distilled water) to get rid of dirt and other dust particles. Using oven, the leaves were dried and grounded into fine powder using motor and pestle. The whole powdered drug was put in a stoppered container with the respective solvents (Methanol, ethanol, ethyl acetate and n hexane) and kept at RT for a period of 2-3 days with frequent agitation. Whatman filter paper no 1 is used to filter the mixture. The filtrate obtained was evaporated until the concentrated extract is obtained. Rotary evaporator is used for evaporation of plant extracts.

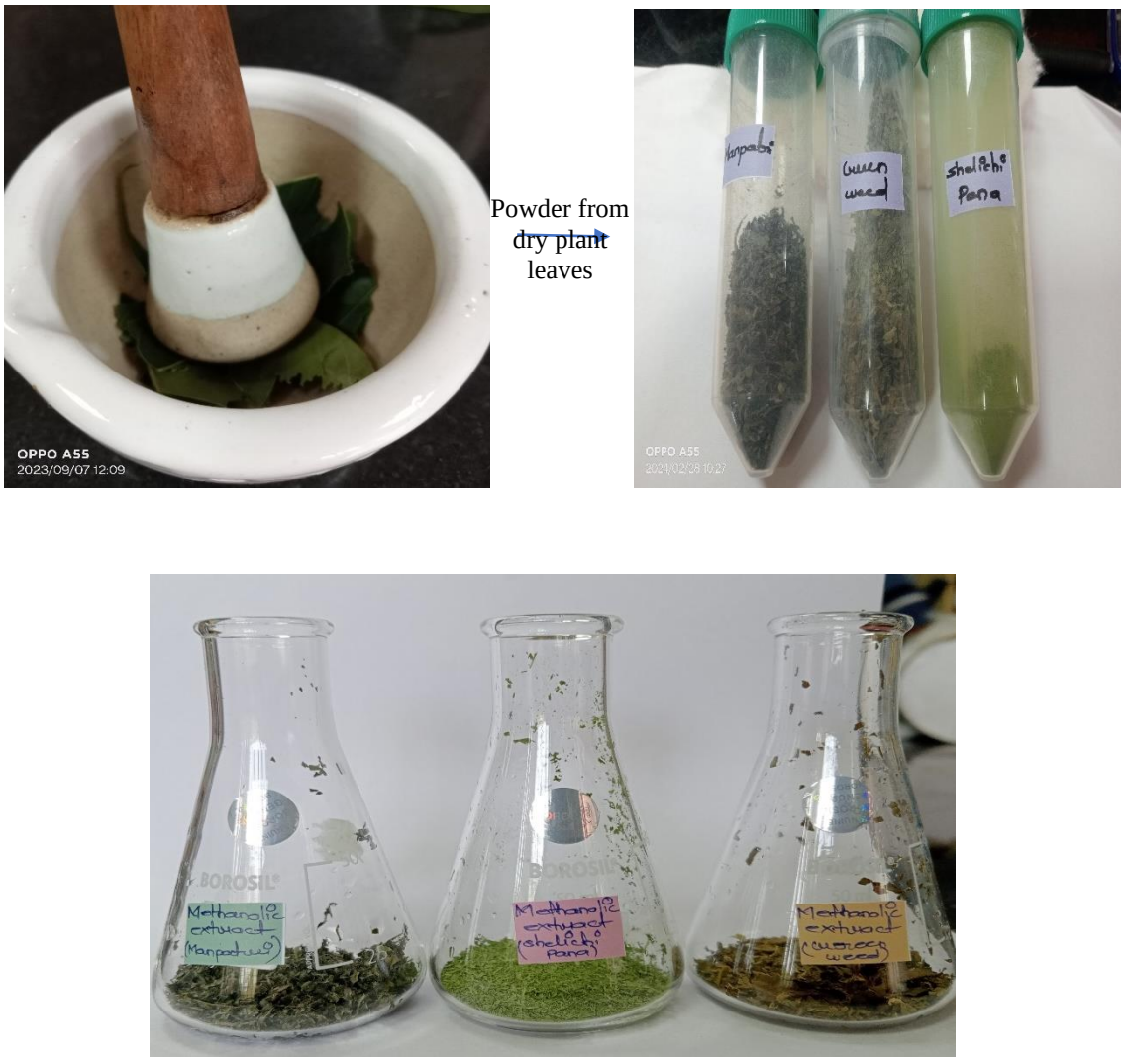
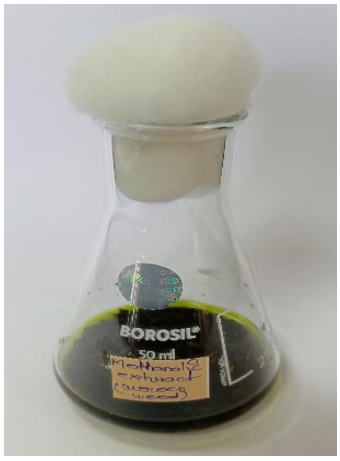


Figure 3 .2.1 Dried plant leaves crushed into fine powder using motor and pastel

a

b

c



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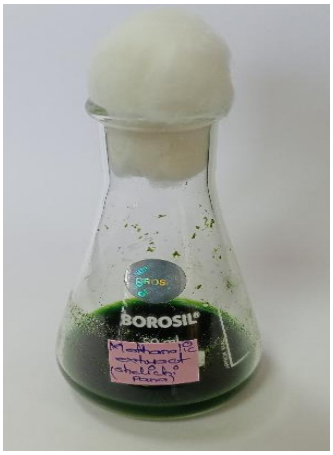

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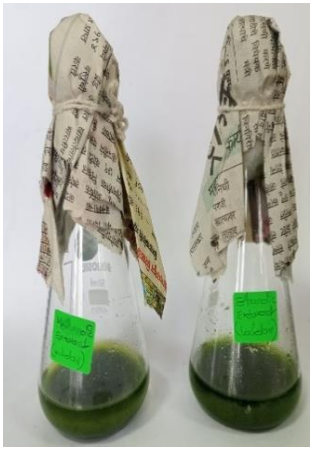
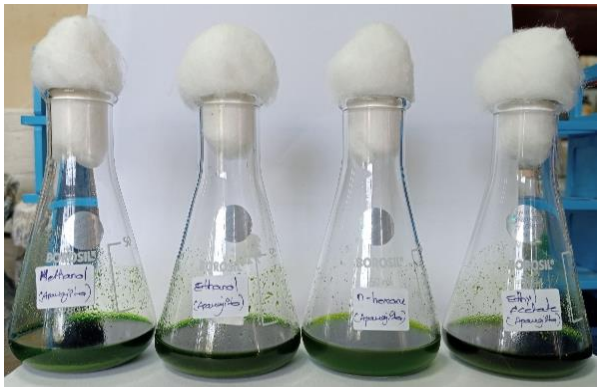
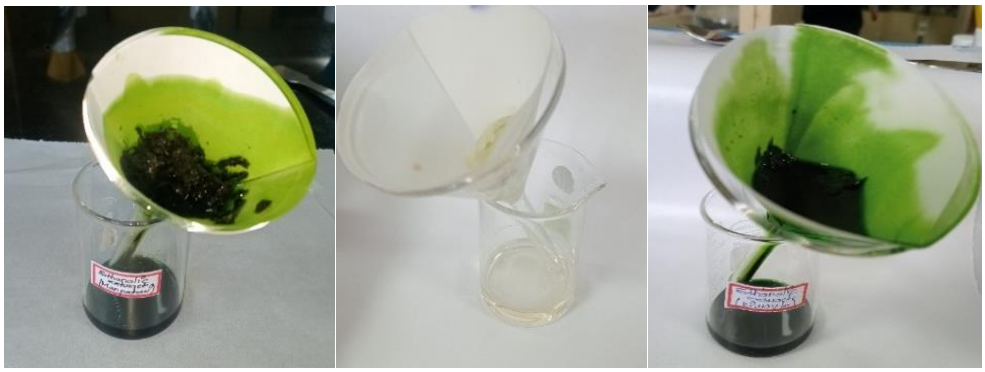


f



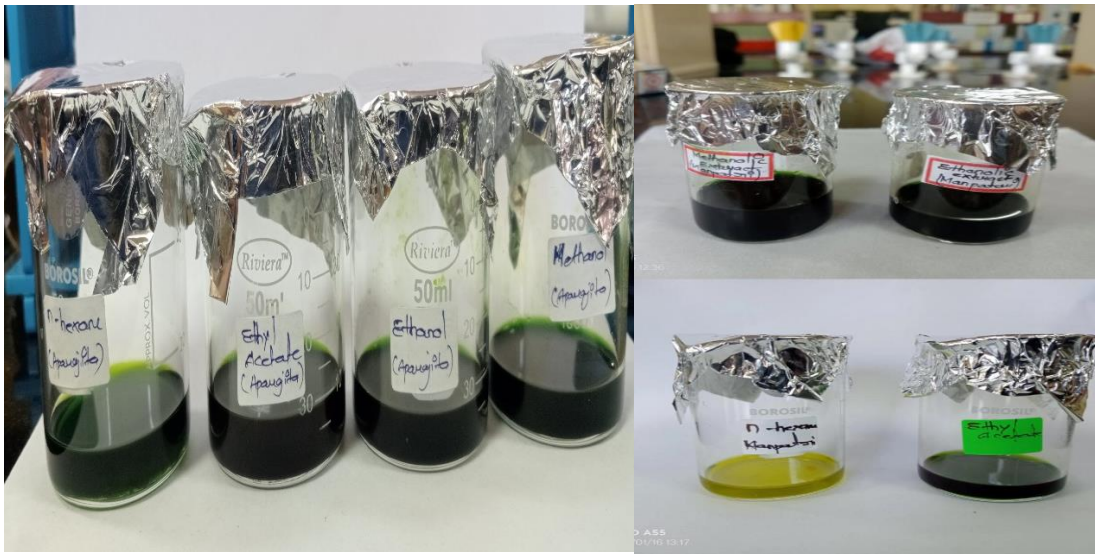
g

Figure 3.2.2. Dried leaf powder susupended in different solvents for extraction of bioactive compounds.a)Methanolic and Ethanolic extract of *A.parviflora* b)Methanolic and Ethanolic extract of *A. paniculata* c)Methanolic and ethanolic extract of *C. melo* d)Methanolic extract *of S.trilobata* e)Methanolic extract of *P.cineraria* e)Methanolic and ethanolic extract of *P amboinicus* g)Methanolic, Ethanolic, N-hexane and ethyl acetate extract of *C.ternatea*



a b c

Figure 3.2.3 Filtration of extract using whatmann filter paper no 1 to remove suspended particles.Filtration of a) Methanolic extract *A. parviflora* b)Methanolic extract of *C. melo* c)Methanolic extract of *A. paniculata*



a b

Figure 3.2.4 Filtered plant extract kept at R.T for concentration by evaporation.N-hexane,ethyl acetate, ethanol and methanol extract of a)*C.ternatea* and b) *A.parviflora*.

Figure 3.2.5 Extracts stored in eppendorf tubes which was further used for determining Antibacterial/QQ activity and phytochemical analysis.

3.3 Test organisms

The extracts of plants were tested against 4 pathogenic organisms for antibacterial and Quorum quenching. The standard pathogenic bacterial strains include one Gram-positive bacteria (*Streptococcus pyogens* ATCC 19615) and three Gram-negative bacteria (*Salmonella typhimurium* ATCC 14028, *Serratia marcescens* and *Chromobacterium violaceum*).

3.4 Determination of Antibacterial and Quorum quenching activity of plant extracts by Agar well diffusion method

a) Antibacterial assay:

i)Test microorganisms and growth media:

Antibacterial activities of the medicinal plant extracts were determined against standard pathogenic bacterial strains of both Gram-positive (*Streptococcus pyogens* ATCC 19615) and Gram-negative (*Salmonella typhimurium* ATCC 14028) bacteria. Nutrient agar (pH. 7.4)/Mueller Hinton agar (pH. 7.2-7.4) (Appendix I) is used as the culture medium of choice to check antibacterial activity.

Method:

The bacterial cultures were inoculated in NB broth and incubated for 18 hours in incubator at 37°C. After 18 hours, bacterial suspensions were prepared by diluting the organisms in sterile 0.85% saline. For bacterial growth, 1ml of the suspension of each test organisms was inoculated in 20ml nutrient agar tubes. The sterile seeded nutrient agar was poured in sterile Petri plates and Plates were left undisturbed for 10 minutes to let the plates solidify. Wells of the size 6mm were punched using sterile corkborers into the nutrient agar (NA) plates. Using sterile tips ,50 micro liters of the extract of each plant was put into wells on all plates. Plates were kept for 30 minutes for pre-diffusion in refrigerator. After incubation at 37°C, the sizes of zone of inhibition (in mm) were measured. Respective solvents were used as a control.

b) Quorum quenching activity

i)Test Microorganisms

The two pathogenic strains of Gram-negative bacteria (*serratia* marcescens and *Chromobacterium violaceum*) were tested for Quorum quenching activity by Agar well diffusion method.

Method:

The bacterial cultures were inoculated in NB broth and incubated at 37°C for 18 hours and used for QQ activity. After 24 hours, bacterial suspensions were prepared by diluting the organisms in sterile 0.85 % saline. For bacterial growth, 1ml of the suspension of each test organism was inoculated in 20ml nutrient agar tubes. The sterile seeded nutrient agar was poured in sterile Petri plates. Plates were left undisturbed for 10 minutes to let the plates solidify. Wells of the size 6mm were punched using sterile corkborers into the nutrient agar plates. Using sterile tips ,50 micro liters of the extract of each plant was put into wells on all plates. Plates were kept for 30 min. for pre-diffusion in refrigerator. After incubation at 37°C,

the different zones of pigment inhibition were measured. Respective solvents were used as a control.

3.5 Qualitative determinations of phytochemical constituents in medicinal plant extracts

To check which metabolite may be responsible for QQ and antibacterial activity we analyzed plant extracts for phytochemical composition.

a) Detection of Alkaloids (Pradeep et al. 2014)

i)Wagner's test

In sterile test tube 1 ml of plant extract was taken. Then 1 ml of Wagner's reagent (Appendix I) was added and shaken. Appearance of reddish-brown precipitate shows the presence of alkaloids.

b) Test for Glycosides

2 ml of the leaf extract was added to 3ml of chloroform and 1 ml of 10% ammonium solution (Appendix I) was added. Presence of glycosides were indicated by formation of pink colour.

c)Test for Tannins

i) Gelatin test

To 1 ml of plant extract (1% solution of gelatin were added to solution containing 10% sodium chloride) (Appendix I) add. Presence of tannins were indicated by formation of white color precipitate.

d) Test for Terpenoids

i)Salkowski's test

5 ml extract was dissolved in 2 ml chloroform and then 3 ml concentrated sulphuric acid was added to the solution. Formation of reddish brown colored at interface showed the presence of terpenoids.

e) Test for Phenols

i)Ferric chloride test

1 ml of extract was treated with 3-4 drops of 10% ferric chloride solution (Appendix I).

Presence of phenols were indicated by formation of deep blue or black or dark green color

f) Test for Flavonoids

i)Alkaline reagent test

In sterile test tube 1 ml of medicinal plant extract was taken. To that few drops of 10% NaOH solution (Appendix I) were added and shaken. Emergence of intense yellow color which turns colorless after addition of dilute hydrochloric acid implies the existence of flavonoids.

g) Test for Saponins

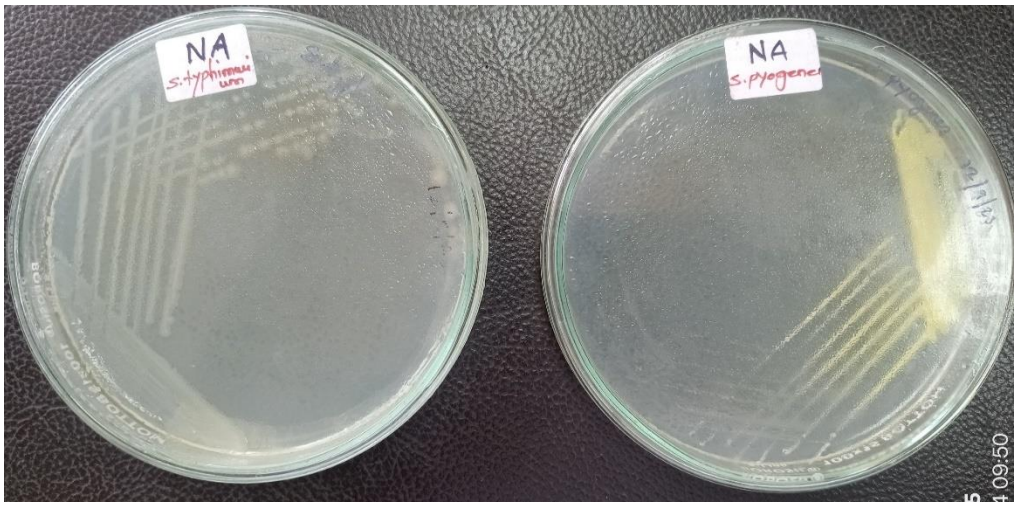
To 0.5 ml of plant extract add 0.5 NaoH and 1 ml ethanol. Shake and keep in water bath for 15 minutes and then add 3 ml of water and shake vigorously. Appearance of foamy solution indicates presence of saponins.

4.
Result and Discussion

4.1 Determination of Antibacterial Activity (AA)

4.1.1 Test organism

The standard pathogenic bacterial strains used to evaluate antibacterial activity of all seven medicinal plants include one Gram-positive (*Streptococcus pyogens* ATCC 19615) and one Gram-negative (*Salmonella typhimurium* ATCC 14028) bacteria.

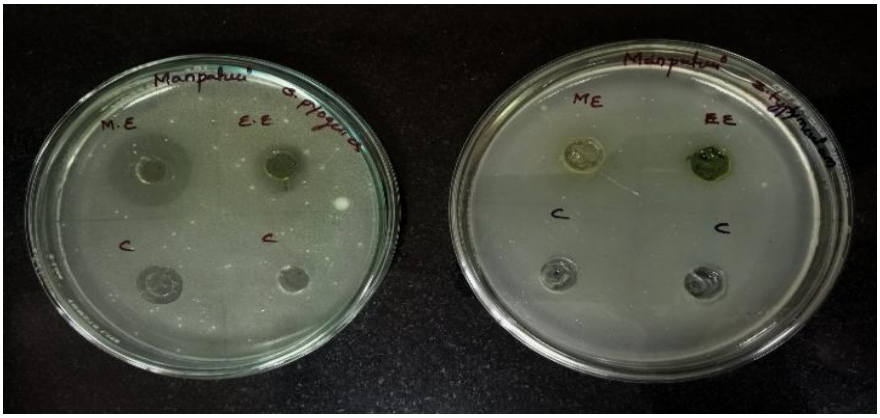


a b

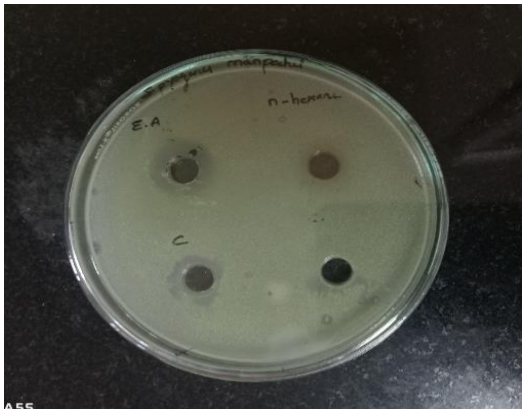
Figure 4.1.1.1 Tested standard pathogenic bacterial strains a) *S. typhimurium* ATCC 14028 b) *S. pyogens* ATCC 19615

4.1.2 Antibacterial assay

Plant extracts of seven medicinal plants were screened for Antibacterial Activity (AA) against two pathogenic bacterial strains *Streptococcus pyogens* ATCC 14028 and *Salmonella typhimurium* ATCC 19615. Out of the seven plant extracts, the methanolic extracts (ME) of (*A.parviflora, A. paniculate, P. cineraria* and *S. trilobata*), ethanolic extract (EE) of (*A.parviflora, A. paniculate*) and ethyl acetate extract of (*A. parviflora, S. trilobata*) showed inhibitory zone against *S. pyogens*. No inhibitory zone were observed for the extracts of (*C. ternatea C. amboinicus C. melo L.*) against *S.pyogens*. Only the EA (ethyl acetate) extract of *S. trilobata* plant showed zone of inhibition when screened against *S. typhimurium*.



a c

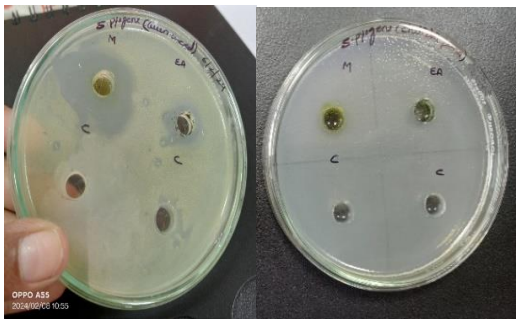


b

Figure 4.1.2.1 Methanolic (ME), Ethanolic (EE) and Ethyl acetate (EA) plant extracts of *A. parviflora* showing zone of inhibition against a) and b) *S. pyogens* c) No zone of inhibition against *S. typhimurium.*

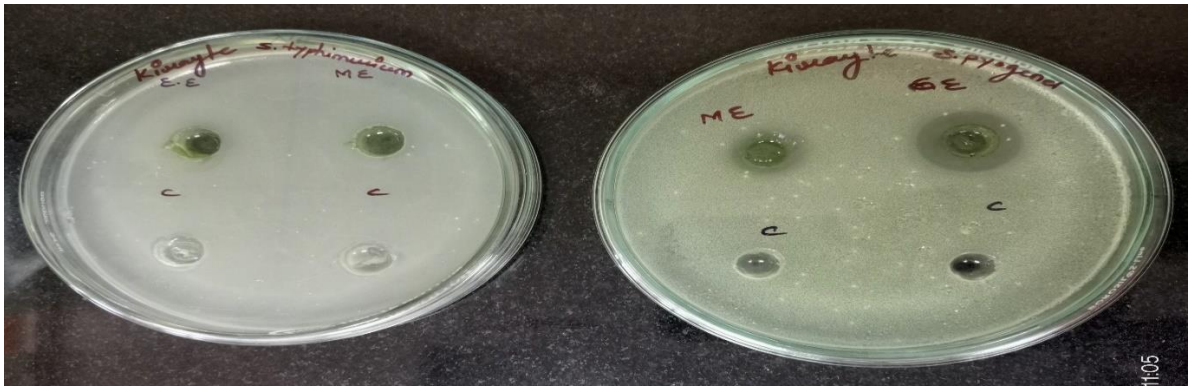
a b

Figure 4.1.2.2 Methanolic (ME) plant extract of *P. cineraria* showing zone of inhibition against a) *S. pyogens* and b) *S. typhimurium*



a b

Figure 4.1.2.3 Methanolic (M) and Ethyl acetate extract of *S. trilobata* showing a) Zone of inhibition against *S. pyogens* b) No zone of inhibition against *S. typhimurium*

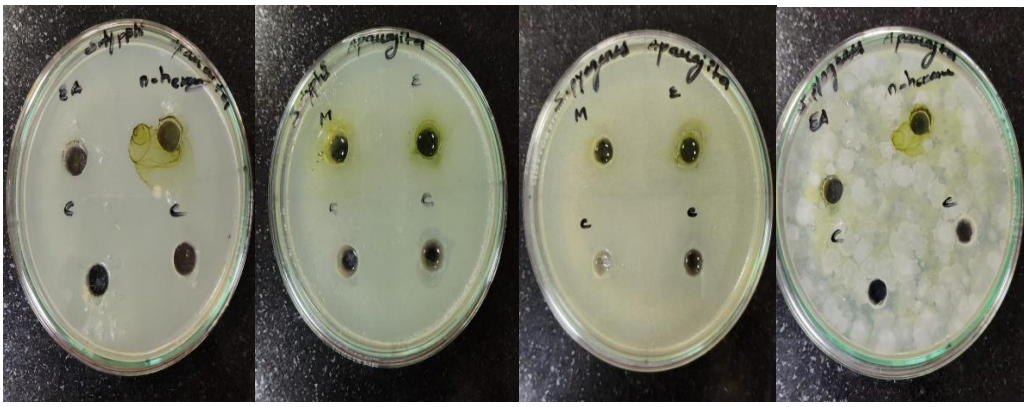


a b

Figure 4.1.2.4 Methanolic (ME) and Ethanolic (EE) extract of *A. paniculata* showing a) No zone of inhibition against *S. typhimurium* b) Zone of inhibition against *S. pyogens*

a b

Figure 4.1.2.5 Methanolic (ME) and ethanolic (EE) extracts of a)*C. melo.L* and b)*C.amboinicus* showing no zone of inhibition againt *S.pyogens* and *S. typhimurium*.



a b

Figure 4.1.2.6 Methanolic (M), Ethanolic (E), Ethyl acetate (EA) and N-hexane extract of *C. ternatia* showing no zone of inhibition against a) *S. typhimurium* and b) *S. pyogens*

Plant Extracts	Zone of Inhibition (in mm)					
	S. pyogens ATCC 19615			*S. typhimurium* ATCC 14028		
	Methanol	Ethanol	Ethyl acetate	Methanol	Ethanol	Ethyl acetate
A. parviflora	22 mm	11 mm	1.6 mm	-	-	-
C. ternatia	-	-	-	-	-	-
A. paniculata	10 mm	20 mm	-	-	-	-
P. cineraria	24 mm	-	14 mm	-	-	18 mm
C. amboinicus	-	-	-	-	-	-
S. trilobata	23 mm	-	-	-	-	-
C. melo L.	-	-	-	-	-	-

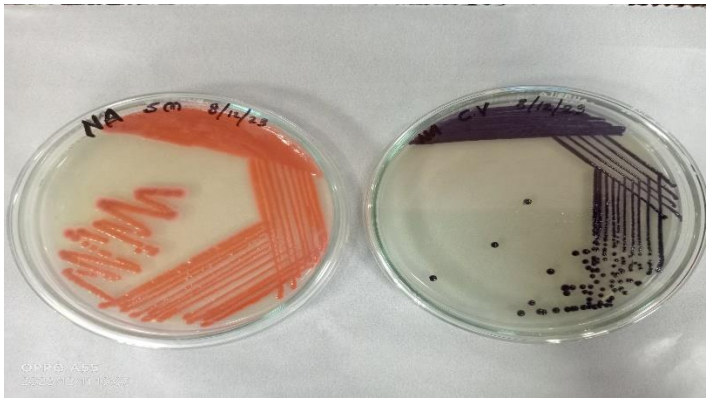
Key: - No zone of inhibition

Table 2: Zone of inhibition (in mm) by all seven medicinal plants

4.2 Determination of Quorum quenching activity

4.2.1 Test Organism

The pathogenic strains used for testing Quorum quenching activity of all seven medicinal plants include two Gram-negative bacteria *(serratia marcescens* and *Chromobacterium violaceum*).

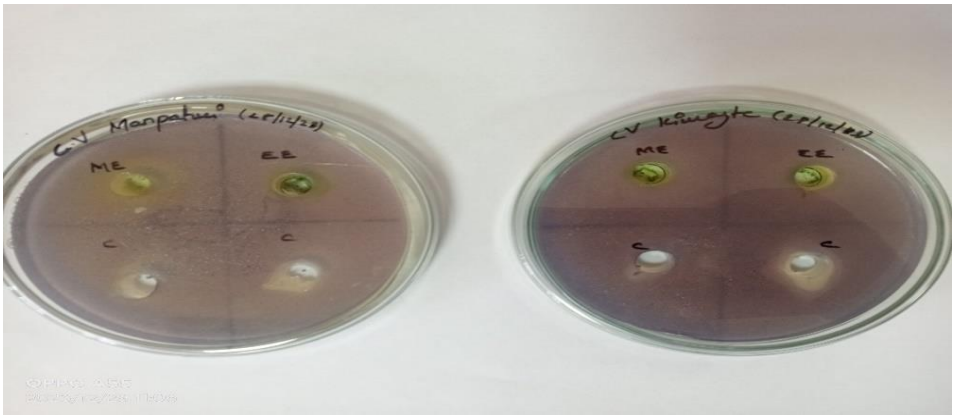


a b

Figure 4.2.1.1 Tested pathogenic bacterial strains a) *serratia marcescens* b) *Chromobacterium violecium*

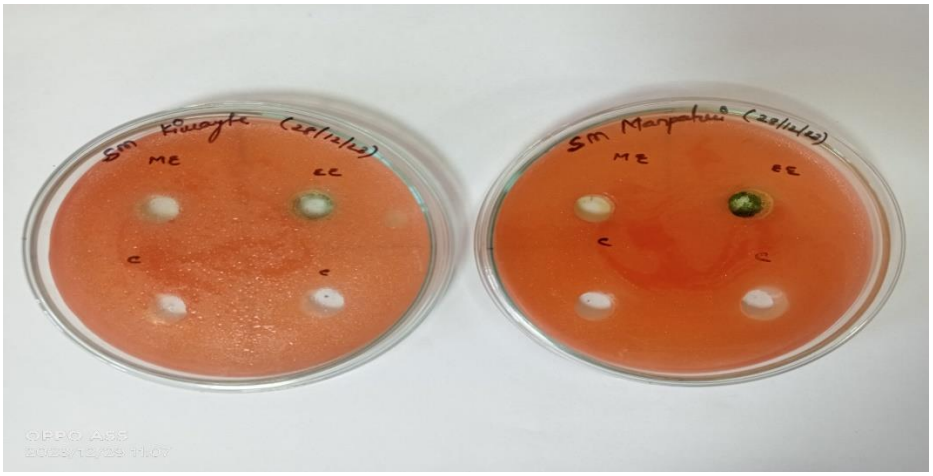
4.2.2 Quorum quenching activity

The plant extracts of seven different medicinal plants were screened for Quorum quenching against two pathogenic bacterial strains. Out of which, the ethyl acetate and methanolic leaf extracts of *P. cineraria* plant showed both zone of pigment inhibition against both the strains. The M (methanolic) extract of *P. cineraria* showed zone of pigment inhibition against both the strains. The methanolic and ethyl acetate extract of *S. trilobata* showed only zone of pigment inhibition against both bacterial strains. No zone of pigment inhibition was observed for remaining five plants.



a b

Figure 4.2.2.1 Methanolic (ME) and Ethanolic (EE) extracts of a) *A. parviflora* and b) *A. paniculat*a showing no pigment inhibition against *Chromobacterium violeceum.*



a b

Figure 4.2.2.2 Methanolic (MM) and Ethanolic (EE) extracts of)*A. paniculata* and b)*A. parviflora* showing no pigment inhibition against *Serratia marcescens*

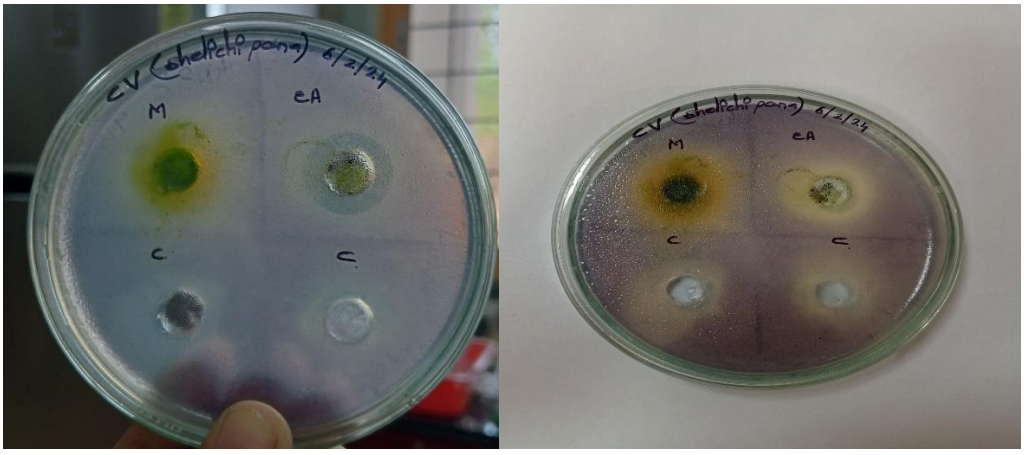


Figure 4.2.2.3 Methanolic (M) and Ethyl acetate (EA) extracts of *P. cineraria* showing Pigment inhibition against *Chromobacterium violeceum* without inhibiting growth.

Figure 4.2.2.4 Methanolic (M) and ethyl acetate (EA) extract of *P. cineraria* showing inhibition of pigment of *S.marcescens* without inhibiting growth of *S.marcescens.*

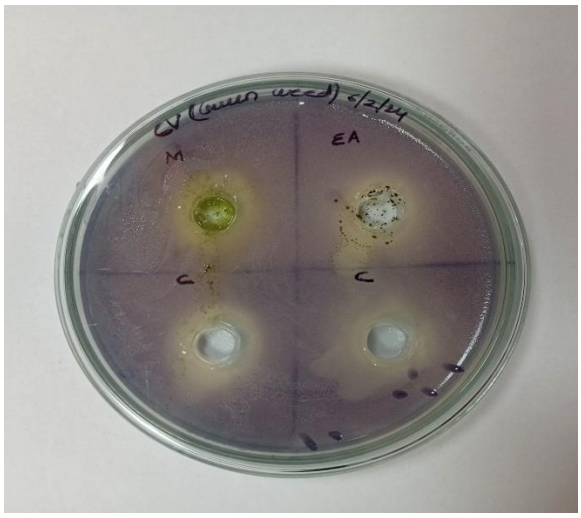
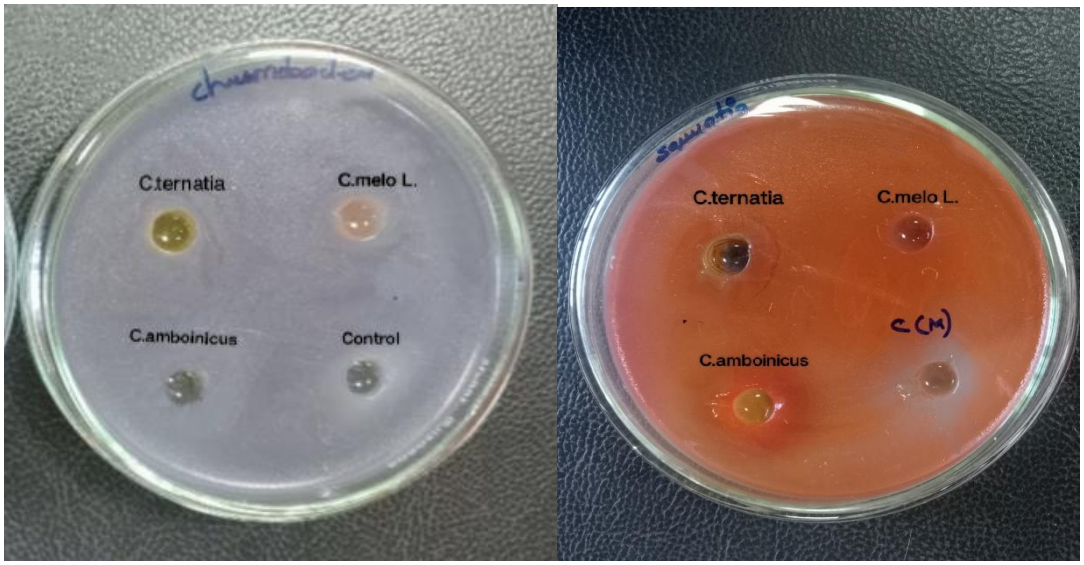


Figure 4.2.2.5 Methanolic (M) and Ethyl acetate (EA) extract of *S. trilobata* showing pigment inhibition against *Chromobacterium violeceum* without growth inhibition.

Figure 4.2.2.6 Ethyl acetate (EA) extract of *S.trilobata* showing pigment inhibition against *S. marcescens* without growth inhibition



a b

Figure 4.2.2.7 Methanolic extracts of *C. ternatia, C. melo* L. and *C. amboinicus* showing no pigment inhibition against a) *C. violaceum* and b) *S. marcescens*.

Plant Extract		Pigment inhibition In mm	
		Methanol	Ethyl acetate
P. cineraria			
Test organism	*C. violaceum*	17 mm	18 mm
	S. marcescens	10 mm	15 mm
S. trilobata			
Test organism	*C. violaceum*	-	14 mm
	S. marcescens	8 mm	10 mm

Key: - No pigment inhibition

Table.3: Zone of pigment inhibition in mm

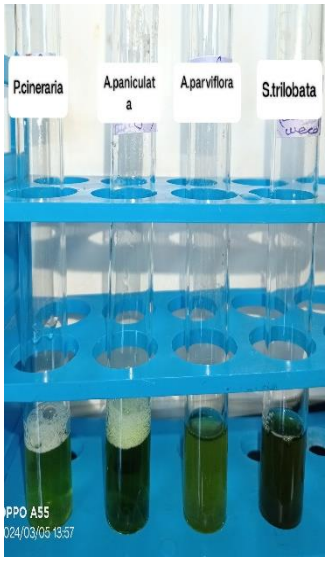
4.3 Qualitative determination of phytochemical constituents in plant extracts

The phytochemical characteristics of four medicinal plants tested were summarized in table no. 4.5.1a. It could be seen that alkaloids and phenols were present in the leaf extracts all four plants. Leaf extracts of *S.trilobata* and *P.cineraria* showed presence of tannins. Glycosides were present only in *P. cineraria* leaf extract. Terpenoids were present in extracts of *A. paniculata, S. trilobata* and *P. cineraria*. Saponins were present in leaf extracts of *A. panicilulata* and *P. cineraria*. All four plants showed absence of flavonoids.

a

b

c



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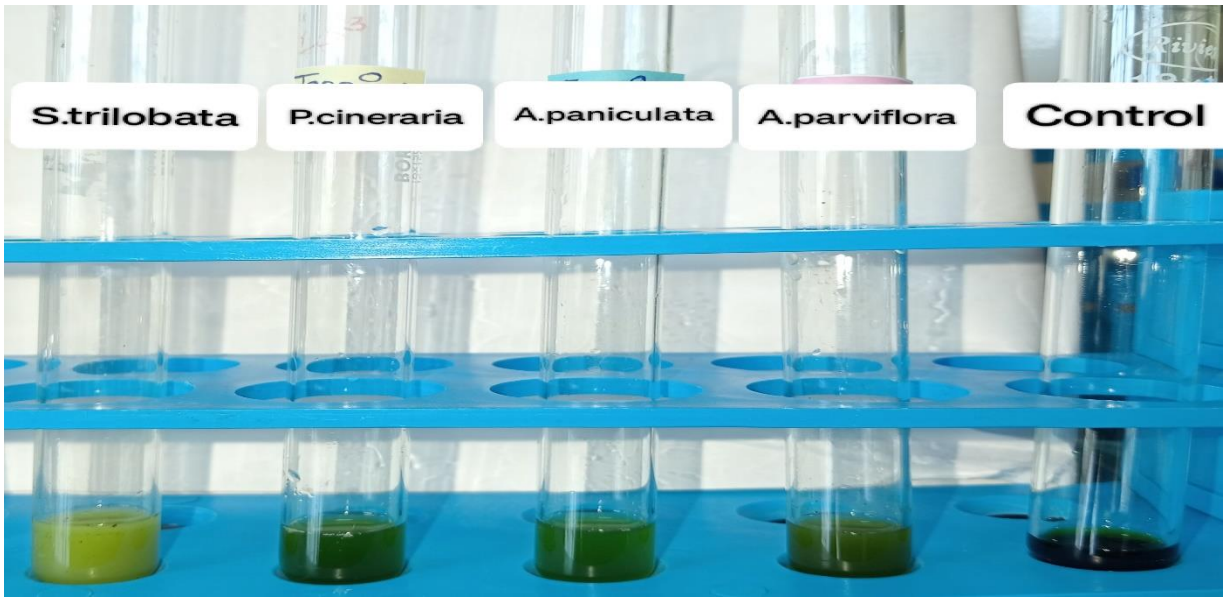

g

Figure 4.3.1 Plants extracts of *A. parviflora*, *A. paniculate*, *P. cineraria* and *S. trilobata* tested for phytochemical constituents a) Alkaloids b) Phenols c) Glycosides d) Saponins e) Terpenoids f) Flavonoids g) Tannins.

Plant extracts	Phytochemical tests						
	Tannins	Glycosides	Terpenoids	Phenols	Alkaloids	Flavonoids	Saponins
A.parviflora	-	-	-	+	+	-	-
A.paniculata	-	-	+	+	+	-	+
S. trilobata	+	-	+	+	+	-	-
P. cineraria	+	+	+	+	+	-	+

Key: + present - Negative

Table 4: Phytochemical Analysis

Discussion:

Plant extracts	Antibacterial activity						Quorum quenching activity			
	S. pyogens Zone of inhibition (In mm)			*S. typhimurium* Zone of inhibition (In mm)			*C. violeceum* Pigment inhibition		*S. marscescens* Pigment inhibition	
	Methanol	Ethanol	EA	Methanol	Ethanol	EA	Methanol	EA	Methanol	EA
A.parviflora	22 mm	11 mm	1.6 mm	-	-	-	-	-	-	-
C. ternatea	-	-	-	-	-	-	-	-	-	-
A.paniculata	10 mm	20 mm	-	-	-	-	-	-	-	-
P. cineraria	24 mm	-	14 mm	-	-	18 mm	17 mm	18 mm	10 mm	15 mm
C.amboinicus	-	-	-	-	-	-	-	-	-	-
S.trilobata	23 mm	-	-	-	-	-	-	14 mm	8 mm	10 mm
C. melo L.	-	-	-	-	-	-	-	-	-	-

Key: - No zone of inhibition and pigment inhibition

Table 5: Zone of inhibition and pigment inhibition in mm by all seven plants

The plants extract of seven different medicinal plants were screened for antibacterial activity against two pathogenic standard bacterial strains of *S. pyogens* ATCC 19615 and *S. typhimurium* ATCC 140*28*. Out of the seven plants, the ME (methanolic extract) of *A. parviflora, A. paniculate, P. cineraria and S. trilobata* showed inhibitory zones ranging from 22 mm, 10 mm, 24 mm and 23 mm respectively against Gram-positive bacteria *S. pyogens*. The EE (ethanolic extract) of *A. parviflora* and *A. paniculate* showed inhibition zones ranging from 11 mm and 20 mm respectively. against *S. pyogens*. The Ethyl acetate extract of *A. parviflora* and *P. cineraria* showed inhibition zones of 1.6 mm and 14 mm respectively. against Gram negative bacteria. Only the ethyl acetate (EA) extract of *S. trilobata* showed

zone of inhibition of diameter 18 mm when screened against the Gram-negative strain of *S. typhimurium*. The extracts prepared in methanolic solvent showed largest zone of inhibition as compared to the other solvents against both the tested organisms. Therefore, from our results we can assume that the methanol can efficiently extract a wide array of bioactive compounds from plants which are responsible for the antibacterial potential of these plants. These results also indicates that the plant extracts we tested in our studies were more effective against the Gram-positive bacteria as compared to the Gram-negative bacteria.

Similarly, plant extracts were tested for Quorum quenching activity against *C.violeceum* and *S. marcencens*. Out of which the ethyl acetate and methanolic extracts of *P. cineraria* showe*d* zone of pigment inhibition of diameter (18 mm and 17 mm) and (15 mm and 10 mm) against *C. violeceum* and *S. marcencens* resp. *S. trilobata* showed zone of pigment inhibition of diameter (14 mm,10 mm and 8 mm) against both the tested organisms. These medicinal plant extracts when subjected to the phytochemical analysis showed presence of various bioactive compounds like tannins, terpenoids, phenols, alkaloids etc. Therefore, from our results we can say that these compounds can interfere with the Quorum quenching system of tested bacteria disrupting their communication and potentially reducing the virulence.

Referring table, no 5 it is evident that extracts of *P cineraria* (methanol and ethanol) showed best QQ activity against both pathogens as compared to *S. trilobata*. Also, it was observed that *P cineraria* extracts showed larger inhibitory zones against both pathogens (*S. pyogens* and *S. typhimurium*.) as compared to other plants. Also, when we analyzed phytochemically it was clear that *P. cineraria* methanolic extract shows presence of Tannins, terpenoids, phenols, alkaloids and saponins which reveals that both highest antibacterial and QQ activity is due to the presence of phytochemicals.

5.
CONCLUSION

Based on the result discussed above, it can be concluded that plant extract has significant promise as an antibacterial and quorum quenching agents against the tested standard pathogenic strains of bacteria (*S. pyogens* ATCC 19615, *S. typhimurium* ATCC 14028, *C. violeceum and S. marcescens*). Out of the 7 plants, the *P. cineraria* plant showed highest antibacterial and quorum quenching potential. This potential of *P. cineraria* plant can be because of the presence of various phytochemical compounds in this plant. These compounds comprise of phenols, alkaloids, terpenoids, glycosides, saponins, flavonoids etc. Therefore, presence of all these compounds in *P. cineraria* plant ensures that it can be useful in various medicinal preparations for treating infectious disorders brought on by microbes that have developed resistance.

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APPENDIX I

MEDIA COMPOSITION

1. Nutrient Agar (NA)

Ingredients	Grams/liter
Peptone	50
Yeast extract	10
Sodium chloride	5
Distilled water	1000
Agar	15

Final pH: 7.4

2. Mueller Hinton Agar (MHA)

- Mueller Hinton Agar is a standardized solid medium recommended for the study of the susceptibility of bacteria to antimicrobial agents by the method of diffusion (Kirby-Bauer method) or dilution in agar. It is primarily used for testing the antimicrobial susceptibility of an organism. It aids in the cultivation of Neisseria. It can be used for food testing, especially in procedures that involve aerobic and facultative anaerobic bacteria.

Ingredients	Grams/liter
Peptone	17.5
Meat extract	2
Starch	1.5
Agar	17
Final pH	7.3 +/- 0.1

APPENDIX II

Reagents for phytochemical analysis

1)Wagner's reagent

Ingredients	**g/100ml**
Iodine	2
Potassium iodide	6
Distilled water	100ml

2) 10% Ammonium solution

Ingredients	
Ammonium solution	1.6 ml
Distilled water	100ml

3)10% Sodium chloride solution

Ingredients	**g/100ml**
Sodium chloride	10
Distilled water	1oo ml

4) Gelatin test

Prepared by dissolving 1g gelatin in 10% sodium chloride solution under warm condition (Always prepared freshly).

Ingredients	**g/100 ml**
Gelatin	1
Distilled water	100 ml

5)10% ferric chloride solution

Ingredients	**g/100 ml**
Ferric chloride	10
Distilled water	100ml

10% Sodium hydroxide solution

Ingredients	**g/100 ml**
Sodium hydroxide	10
Distilled water	100 ml

Note: All reagents need to be freshly prepared for phytochemical analysis

SUMMARY

BIOPROSPECTING OF MEDICINAL PLANTS FROM GOA FOR THEIR ANTIBACTERIAL AND QUORUM QUENCHING ACTIVITY

Emergence of antibiotic resistant pathogens has become a serious health issue and it also threatens the prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. 70% of the bacterial infections are Multi Drug Resistant (MDR) and this affect country's economy and health. Since antibiotics are costly and it puts tremendous pressure on middle class and poor families a cheap and reliable alternative is need of an hour. Medicinal plants are the richest source of chemical compounds and the use has increased rapidly due to their antibacterial, antioxidant activities and low toxicity. Organic solvent extracts of leaves and bark of medicinal plants reveals promising antibacterial activity on bacterial pathogens. Therefore, bioprospecting of medicinal plants from Goa for antimicrobial and quorum quenching activity can be a cheap and reliable alternative to treat MDR infections.

This project work was therefore undertaken to study the antibacterial and Quorum quenching (QQ) potential of different solvent extracts of selected medicinal plants. This study includes selection of easily available medicinal plants from Goa, preparation of their extracts using different organic solvents, then screening this medicinal plant extracts for their antibacterial and quorum quenching potential and then to check which bioactive compounds present in plants may be responsible for their antibacterial and QQ potential these extracts were subjected to qualitative phytochemical analysis.

Seven different medicinal plants like Artemesia. parviflora (Manpatri), Andrographis. paniculate (Kirayte), Clitoria. ternatea (Aprajita) ,Coleus. Amboinicus (Vatelav), Sphagneticola. trilobata (Yellow creeping daisy) , Prosopis Cineraria (Shami plant) and Cucumis. melo L (Karit) were collected. These fresh plants were then washed separately using distilled water. After washing, leaves were dried in an oven and extracts were prepared

using Maceration method. The extracts were prepared using four different solvents like Methanol, Ethanol, Ethyl acetate and n-hexane. These prepared plant extracts were screened against the standard pathogenic bacterial strains of S. pyogens ATCC 19615, S. typhimurium ATCC 14028, C. violaceum and S. marcescens for their antibacterial and QQ potential respectively. The plants which show best antibacterial and QQ potential were then subjected to phytochemical analysis to check which metabolites in plants were responsible for their antibacterial and QQ potential.

Plant extracts	Antibacterial activity						Quorum quenching activity			
	S. pyogens Zone of inhibition (In mm)			*S. typhimurium* Zone of inhibition (In mm)			*C. violeceum* Pigment inhibition		*S. marscescens* Pigment inhibition	
	Methanol	Ethanol	EA	Methanol	Ethanol	EA	Methanol	EA	Methanol	EA
A.parviflora	22 mm	11 mm	1.6 mm	-	-	-	-	-	-	-
C. ternatea	-	-	-	-	-	-	-	-	-	-
A.paniculata	10 mm	20 mm	-	-	-	-	-	-	-	-
P. cineraria	24 mm	-	14 mm	-	-	18 mm	17 mm	18 mm	10 mm	15 mm
C.amboinicus	-	-	-	-	-	-	-	-	-	-
S.trilobata	23 mm	-	-	-	-	-	-	14 mm	8 mm	10 mm

C. melo L.	-	-	-	-	-	-	-	-	-	-

Key: - No zone of inhibition and pigment inhibition

Table 5: Zone of inhibition and pigment inhibition in mm by all seven plants

Plant extracts	Phytochemical tests						
	Tannins	Glycosides	Terpenoids	Phenols	Alkaloids	Flavonoids	Saponins
A.perviflora	-	-	-	+	+	-	-
A.paniculata	-	-	+	+	+	-	+
S. trilobata	+	-	+	+	+	-	-
P. cineraria	+	+	+	+	+	-	+

Key: + positive - negative

Table no 2: Phytochemical analysis

Seven medicinal plants were screened for antimicrobial activity against two pathogenic bacterial strains S. pyogens and S. typhimurium. Out of the seven plants, the methanolic extract of A. parviflora, A. paniculate, P. cineraria and S. trilobata showed inhibition zones ranging from 22 mm, 10 mm, 24 mm and 23 mm respectively against Gram-positive bacteria S. pyogens. The ethanolic extract of A. parviflora and A. paniculate showed inhibition zones ranging from 11 mm and 20 mm respectively. against S. pyogens. The Ethyl acetate extract of A. parviflora and P. cineraria showed inhibition zones of 1.6 mm and 14 mm respectively. against Gram-negative bacteria. Only the ethyl acetate (EA) extract of S. trilobata showed zone of inhibition of diameter 18 mm when screened against the Gram-positive strain of S. typhimurium. The extracts prepared in methanolic solvent showed largest zone of inhibition as compared to the other solvents against both the tested organisms.

Therefore, from our results we can say that the methanol can efficiently extract a wide range of bioactive compounds from plants which are responsible for the antibacterial potential of these plants. These results also indicates that the plant extracts we tested in our studies were more effective against the Gram-positive bacteria as compared to the Gram-negative bacteria.

Similarly, all the seven-plant extract were screened for Quorum quenching activity against C. violaceum and S. marcescens. Out of which the ethyl acetate and methanolic extracts of P. cineraria showed zone of pigment inhibition of diameter (18 mm and 17 mm) and (15 mm and 10 mm) against C. violaceum and S. marcescens resp. S. trilobata showed zone of pigment inhibition of diameter (14 mm,10 mm and 8 mm) against both the tested organisms. These plant extracts when subjected to the phytochemical analysis showed presence of various bioactive compounds like tannins, terpenoids, phenols, alkaloids etc. Therefore, from our results we can say that these compounds can interfere with the Quorum quenching system of tested bacteria disrupting their communication and potentially reducing the virulence.

Referring table no 1 and 2 it is evident that extracts of P cineraria (methanol and ethanol) showed best QQ activity against both pathogens as compared to S. trilobata. Also it was observed that P. cineraria extracts showed greater inhibition against both pathogens (S. pyogens and S. typhimurium.) as compared to other plants. Also, when we analyzed phytochemically it was clear that P. cineraria methanolic extract shows presence of Tannins, terpenoids, phenols, alkaloids and saponins which reveals that both highest antibacterial and QQ activity is due to the presence of phytochemicals.

Based on the results discussed above, it can be concluded that plant extracts have significant promise as antibacterial and quorum quenching agents against the tested pathogenic strains of bacteria (S. pyogens, S. typhimurium, C. violaceum and S. marcescens). Out of the seven plants, the P. cineraria plant sowed highest antibacterial and quorum quenching potential.

This potential of P. cineraria plant can be because of the presence of various phytochemical compounds in this plant. These compounds comprise of phenols, alkaloids, terpenoids, glycosides, saponins, flavonoids etc. Therefore, presence of all these compounds in P. cineraria ensure that it can be useful in various medicinal preparations for treating infectious disorders brought on by microbes that have developed resistance.