

**ANTIVIRAL AND ANTIMYCOBACTERIAL ACTIVITY OF SELECTED
MEDICINAL PLANTS AGAINST SARS-CoV-2 AND
*MYCOBACTERIUM TUBERCULOSIS***

Submitted in partial fulfillment of the requirements for the award of Master
of Technology Degree in Biotechnology

by

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SATHYABAMA
INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)

Accredited with Grade "A" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the Bonafide work of **ROOPINI K.S** (41810017) who carried out the project entitled "**ANTIVIRAL AND ANTIMYCOBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST SARS-CoV-2 AND MYCOBACTERIUM TUBERCULOSIS**" under our supervision from January 2023 to May 2023

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I ROOPINI K. S (41810017) hereby declare that the Project Report entitled **“ANTIVIRAL AND ANTIMYCOBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANTS AGAINST SARS-CoV-2 AND MYCOBACTERIUM TUBERCULOSIS”** done by me under the guidance of **Dr. Krupakar Parthasarathy (External)** and **Dr.P.Prakash (Internal)** in Centre for Drug Discovery and Development at Sathyabama Institute of Science and Technology is submitted in partial fulfillment of the requirements for the award of Master of Technology degree in Biotechnology.

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ABSTRACT

Mycobacterium tuberculosis and SARS-CoV-2 affect the lungs, interfere with the host immune system, and have similar symptoms. Medicinal plants are naturally available sources, that promote and maintain human health. The secondary metabolites from the medicinal plant can inhibit or slow down the infection caused by the pathogens. So, selected medicinal plants are *Coleus amboinicus*, *Citrus limon*, *Leucas aspera*, *Curcuma longa*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum gratissimum*, *Vitex negundo*, and *Allium sativum*. For the antiviral assays extracts of these plants are prepared by using Soxhlet and the compounds were analysed by GCMS. The obtained compounds were screened for toxicity, and drug-likeness by using insilico methods and then further docked with a docking tool. In Invitro, cytotoxicity studies were carried out. On the other hand, the antimycobacterial activity was analysed by using the Microplate Alamar Blue Assay (MABA). The extracts were also checked for antioxidant, properties, phytochemical screening, haemolytic assay, antibacterial activity, and antimycobacterial activity. The results we obtain that 2 compounds have drug-likeness properties and in cytotoxicity assay, the cells are said to have lower viability when concentration tends to increase. In both antibacterial and antimycobacterial assays the extracts tend to have the inhibition property of bacteria. Hence these plants could be formulated into lead drug molecules by isolating the compounds present in them.

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Chapter 1

INTRODUCTION

1.1 Coronavirus

Coronaviruses are single-stranded positive-sense RNA viruses that are enveloped and have a diameter of 80-180nm. The human coronavirus was first initially discovered in 1965 during the Cold Epidemic and later in 1968, it was compared with other viruses for identifying its family based on morphological differences. These coronaviruses cause colds and infections in the respiratory tract in humans and mammalian domestic animals. In the year 2002-2003 during the winter season coronavirus-related Severe Acute Syndrome (SARS) infections emerged for the first time in Southeast Asian countries. This outbreak resulted in a 10 percent mortality rate and 8000 evident infected people. SARS CoV-1 generally infects humans and mammals; hence, it belongs to the family *betacoronavirus* (Modrow, Falke, et al., 2013). In recent years, the outbreak of the SARS-CoV-2 had created a huge impact globally. When compared to SARS CoV -1, SARS COV-2 doesn't have much mortality rate but its ability to spread is faster than the SARS CoV -1. This virus contains structural and non-structural proteins which are responsible for the host infections. The RNA genome of SARS CoV -2 is present in the Nucleocapsid protein (N protein), and its amino acids interact with the carboxyl end of the membrane protein (M protein). Apart from these, the glycosylated Spike protein (S protein) is present in the club-shaped trimers which are responsible for the host interaction mechanisms, and this protrudes away from the envelope-shaped surface which contains Enveloped protein (E protein). These are the functions of the structural proteins. In non-structural proteins, it includes from Nsp1 to Nsp16 which forms the replicase transcriptome that is responsible for providing multiple enzymes to support structural protein in replication and infections (Gordon et al., 2020; Modrow, Falke, et al., 2013). When correlating 26 viral proteins with 336 human proteins, nearly 16 human proteins have been found to be interacting with the viral proteins. Among 16 proteins, the interaction of the viral protein with the lung tissue has shown a high expression. This proves that the SARS CoV-2 primarily affects the lungs and causes acute respiratory distress syndrome in humans (Gordon et al., 2020).

1.2 Tuberculosis

Tuberculosis is a bacterial infection which is caused by *Mycobacterium tuberculosis* which has infected a quarter of the world's population. In the first stage primary or Ghon complex is formed by inhaling the aerosols containing *Mycobacterium tuberculosis*. After 3 to 8 weeks of inhaling *M. tuberculosis*, it gets disseminated in the lungs through lymph nodes. Before entering the second stage this complex gets converted to the tuberculin reactions. Later this stage lasts for 3 months, during which the haematogenic circulation of bacteria occurs to many organs and other parts of the lungs. At this stage, the acute or fatal disease can occur which results in tuberculosis meningitis or military tuberculosis. The third stage lasts for 3 to 7 months and sometimes it is delayed up to 2 years. In this stage, the bacteria either gets hematogenous dissemination or released into the pleural space. The final stage may take up to 3 years and it slowly develops extrapulmonary lesions in the lungs (Smith, 2003).

So, both *M. Tuberculosis* and *SARS-CoV-2* affect the lungs, interfere with the host immune system, and have similar symptoms. These two lung infections have sometimes been involved in coinfections (Aissaoui et al., 2021).

1.3 Medication for lung infection- Traditional medicinal plants

The medication for these viral and bacterial infections has not been effective to date. The bio compounds function as the secondary metabolites produced by plants and have a primary function in defense mechanism against the virus, mycoplasma, bacteria, and fungi these metabolites are classified into different classes based on their structures, which are Isoprenoids, Polyphenols, and Nitrogen-containing compounds (Korkina & Kostyuk, 2012). So, medicinal plant's naturally available bio compounds have remarkable antiviral potential and effectively treat infections (Akram et al., 2018).

These phytochemicals which were derived from the plants are individually or in a combination with plants by making them into leaf powder, pastes, decoctions,

infusions, and pills (Perera et al., 2021). After extraction of phytocompounds from the plant derivatives, is validated for antiviral activity with various studying models such as computational studies (Kiran et al., 2022a) and preclinical studies (invitro, in vivo) (Perera et al., 2021). The essential oil from the medicinal plants was also analysed for the antimycobacterial assay by using MABA (Microplate Alamar Blue Assay) (Birdi et al., 2012). So, the medicinal plants, *Coleus amboinicus*, *Citrus limon*, *Leucas aspera*, *Curcuma longa*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum gratissimum*, *Vitex negundo*, and *Allium sativum* were chosen to analyze the hindrance of lung infections caused by *M. tuberculosis* and SARS-CoV 2. Hence this work focuses on the following medicinal plant activities against the SARS CoV- 2 and the *Mycobacterium tuberculosis*.

Chapter 2

LITERATURE SURVEY

2.1 VIRUSES AND THEIR CLASSIFICATION

The viruses have a symbiont relationship with the host, which leads to several viral infections. These viral infections lead to several outbreaks of viral diseases which causes a major impact on public health (Roossinck & Bazán, 2017). The mutations that occur in the viral genome are much faster when compared to prokaryotes and Eukaryotes (Koonin et al., 2020). Advanced antiviral drugs are used to treat viral infections. A new era of antiviral drug development has begun since the first antiviral drug, idoxuridine, was approved in June 1963 for the treatment of Herpes Simplex Virus 1 (HSV-1).

Since then, many antiviral drugs have been formulated to treat viral infections (de Clercq & Li, 2016). So, the medicinal plants selected for this review are *Zingiber officinale* Rosc, *Piper longum*, *Syzygium aromaticum*, *Tragia involucrata*, *Anacyclus pyrethrum*, *Andrographis paniculata*, *Hygrophilla auriculata*, *Terminalia chebula*, *Justicia adhatoda*, *Plectranthus amboinicus*(*Coleus amboinicus*), , *Tinospora cordifolia*, *Sida acuta* Burm, *Cyperus rotundus* (Kiran et al., 2022a)*Costus speciosus* (*Saussurea lappa*)(Pandey et al., 2007), *Clerodendrum serratum*, *prema herbacea* (Narayanan et al., 2002)*Barleria prionitis* (Chen et al., 1998a), *Allium sativum*, *Allium cepa*, *Mentha piperita*, *Foeniculum vulgare*, *Ocimum sanctum*, *Origanum vulgare*, *Curcuma longa*, *Cinnamomum cassia*, *Cordyceps militaris* (Kumar Verma et al., 2021), *Vetiveria zizanioides*, *Santalum album*, *Piper nigrum*, *Hedyotis corymbosa*, *Plectranthus vettiveroides*, *Trichosanthes cucumerina* (Srivastava et al., 2021), *Citrus limon* (Khan et al., 2021), *Leucas aspera* (Navabshan et al., 2021a) *Ocimum basilicum* (Chiang et al., 2005)*Ocimum gratissimum* (Gyebi et al., 2021a)and *Vitex negundo*(Alagarasu et al., 2022).

The plants are focused on antiviral property, and it is segregated and tabulated based on the Baltimore classes of viruses. This Baltimore class of viruses consists of seven divisions which are classified based on the structure of the virion's nucleic acid (Koonin et al., 2020) as mentioned below:

CLASS I : dsDNA Genome

CLASS II : ssDNA Genome

CLASS III: dsRNA Genome

CLASS IV : Positive- sense ssRNA

CLASS V : Negative- sense ssRNA

CLASS VI : ssRNA with a DNA intermediate in their growth

CLASS VII: dsDNA with a RNA intermediate in their growth

CLASS VII: dsDNA with a RNA intermediate in their growth

Table 2.1:(The medicinal plants which are selected have antiviral properties for only Class I, IV, V,VI and VII, which includes the different viruses)

CLASSES OF VIRUSES	VIRUS NAME	REFERENCES
CLASS I	1. Herpes simplex virus-1,2 2. Adeno viruses	(Reuven & Weller, 2005), (Minaya et al., 2017),(Ahi & Mittal, 2016)
CLASS IV	1. SARS COV-2 2. Hepatitis A, C 3. Dengue 4. Zika 5. Enteroviruses 6. Coxsackievirus B1	(Marín-Palma et al., 2021; Murugesan & Manoharan, 2020; Quinn et al., 2014; Valenzuela, 1990; White et al., 2016)
CLASS V	1. RSV 2. Influenza virus (H7N3, H9N2, H1N1) 3. Ebola	(Bouvier & Palese, 2008; Collins et al., 2013; Tomar et al., 2019)
CLASS VI	1. HIV- 1,2	("Human Immunodeficiency Virus (HIV)," 2016)
CLASS VII	1. Hepatitis B	(Valenzuela, 1990)

2.2 dsDNA VIRUS

The dsDNA viruses are subdivided into viral families such as Hepadnaviruses, polyomaviruses, Papillomaviruses, Adenoviruses, Herpesviruses, Poxviruses, and Asfarviruses. Among these families, Poxviruses and Asfarviruses cause persistent infections in animals and humans whereas Hepadnaviruses, polyomaviruses, Papillomaviruses, and Herpesviruses cause human cancers. This proves that they have many ways to regulate and improve their division of cells.

2.2.1 HERPES SIMPLEX VIRUS

The Herpes simplex virus comes under the family *Herpesviridae*, subfamily: *Alphaherpesvirus*, genus: *Simplexvirus*, and types: Herpes simplex virus-1 (HSV 1) and Herpes simplex virus-2 (HSV 2) (Modrow, Dietrich, et al., 2013) The medicinal plants which inhibit the HSV viruses are *Terminalia chebula*, *Syzygium aromaticum*, *Cyperus rotundus*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum sanctum*, and *Santalum album*

In *Terminalia chebula*, the compounds chebulagic acid and punicalagin inhibit HSV- 1 surface glycosaminoglycans and glycoproteins by using cell line studies which in turn inactivates viral particles and prevents virus penetration and attachment (Lin et al., 2011). The *T.chebula* also inhibits HSV-2 with the compounds Chebulagic acid and chebulinic acid. Using in vitro studies, these compounds along with the acyclovir were eventually assessed for direct antiviral activity and analysed for post-infection plaque reduction assay. As a result, they have a direct antiviral activity on the virus and have the potency to hinder virus attachment and host penetration (Kesharwani et al., 2017).

Syzygium aromaticum contains compounds Eugeniiin and Eugenol that inhibit the DNA Polymerase enzyme and virus replication site. By that, it prevents viral replication and reduces its infection. This was studied by using infected FL cell lines (El-Saber Batiha et al., 2020; Takechi & Tanaka, 1981).

By using the Bioassay procedure and Endpoint titration technique (EPTT), the Hydro-alcoholic extract from the plant *Cyperus rotundus* have antiviral activity against HSV viruses at a concentration of 500 µg/ml (Dhawan, 2012; Soltan & Zaki, 2009). The peppermint oil from the *Mentha piperita* affects the virus before the host cell penetration and it is also vital against an acyclovir-resistant HSV-1 strain (HSV- 1-ACVres). This was studied by using the plaque reduction assay (Schuhmacher et al., 2003)

Ocimum basilicum contain compounds Ursolic acid and Apigenin are the potential inhibitors of HSV-1 and 2 respectively. The antiviral activity of these compounds is analyzed and their cytotoxicity studies were carried out by using MTT Assay (2,3-Bis[2-methoxy-4-nitro-5-sulfophenyl]-5-[(phenylamino)carbonyl-2H-tetrazolium hydroxide] assay) (Chiang et al., 2005). The sandalwood oil from the *Santalum album* hinders the replication of viruses and this was studied by using a Direct plaque assay and cytotoxicity assay (Benencia & Courrèges, 1999).

2.2.2 ADENOVIRUSES

The Adenovirus comes under the family *Adenoviridae* and Genus: *Mastadenovirus*. They are non-enveloped capsids, with an icosahedral structure exhibiting 20 faces and 12 vertices and a diameter of 80-110 nm) (Modrow, Dietrich, et al., 2013). The plant *Ocimum basilicum* compounds Apigenin and linalool show anti-adenoviral activity against ADV-3, ADV-8, and ADV-11 with the inhibitory effect (EC₅₀ values range from 8.0-23.4 mg/L. Then the compound Ursolic acid hinders the replication of ADV-8 and ADV-11, with EC₅₀ values ranging from 4.2 and 24.5 mg/L (Chiang et al., 2005).

2.3 POSITIVE SENSE ssRNA VIRUS

The single-stranded, positive-sense RNA genome currently contains eight viral families. Viral families with non-enveloped capsids are *Picornaviridae*, *Astroviridae*, *Caliciviridae*, and *Hepeviridae* and Viral families with enveloped capsids are *Flaviviridae*, *Togaviridae*, *Arteriviridae*, and *Coronaviridae*. These viruses have an enzyme called RNA-dependent RNA polymerase (RdRp), which transcribes the RNA positive strand and the complementary negative RNA strand and is formed as

an intermediate product in genome replication. In this study, viruses such as Hepatitis A, Enterovirus, and Coxsackievirus B1(CVB1) belong to the family *picornaviridae*. Virus such as Hepatitis C, Dengue, and Zika belongs to *Flaviviridae*, and the SARS COV-2 belongs to the family *coronaviridae* (Modrow, Falke, et al., 2013)

2.3.1 Hepatitis A virus

The Hepatitis A virus (HAV) belongs to the genus Hepatovirus which has a genome size of 7,478 nucleotides (Modrow, Falke, et al., 2013) This is an orally transmitted disease that affects the individual later in the adulthood stage. The chances of getting this viral infection are around 50 to 80 percent in adulthood whereas in childhood there is no evidence to get infected (Valenzuela, 1990). This HAV generally causes acute liver failure and can be effectively inhibited by using the essential oil extracted from the plant *Foeniculum vulgare*. This was identified by using the invitro and computational studies. In this invitro study, the essential oil from this plant has been tested for its virucidal effect on this virus by using a Plaque reduction assay and its cytotoxic effect was identified by using MTT assay on human lung fibroblast cell line WI38. Concurrently the Hepatitis A virus 3C proteinase inhibition assay was also carried out. This assay is mainly based on the ability of the enzyme (Hepatitis A 3C Proteinase) that cleaves a systemic chromogenic peptide substrate and emits another chromophore. So, the reduction in cleavage of the chromogenic peptide substrate gives the relative inhibitory percentage. As a result of assays, the inhibition was found to be around 66-88% and the IC50 values were from 1.8 to 26.7 $\mu\text{g mL}^{-1}$. Further, this essential oil possesses compounds that were identified by using GC-MS analysis. The obtained compound was studied under *insilico* molecular docking. The compounds such as β -ocimene and β -fenchyl acetate show that these compounds have effective binding in enzyme-binding pockets of the Hepatitis A 3C Proteinase enzyme (Ibrahim & Moussa, 2021a).

2.3.2 ENTEROVIRUSES

Enteroviruses are classified based on their viral genomes, where Enterovirus A, B, C & D are human viruses. The Enterovirus B contains Coxsackievirus A9, B1-B6, Echovirus 1-7, 9, 11-21, 24-27, 29-33, and Enterovirus B69, B73-75, B77-88, B93, B97, B98, B100, B101, B106, B107 (Modrow, Falke, et al., 2013). Among

these viruses, the Enterovirus 71 (E71) and CoxsackievirusB1 (CVB1) are effectively inhibited by the crude and ethanol extracts, extracted from the medicinal plant *Ocimum basilicum*. The purified compounds from these extracts were evaluated against the E71 and CVB1 by using antiviral assays. The compound Ursolic acid was found to be an effective hindrance to the virus replication process and also the dose and time-course effect of ursolic acid against enteroviruses have resulted in the strong likeness between drug concentration and inhibition rate and it also signifies that the drug interferes with the infection and its replication (Chiang et al., 2005).

2.3.3 DENGUE VIRUS

The Dengue virus belongs to the family *Flaviviridae* which contains an mRNA genome that translates into the precursor polypeptides which contain structural and non-structural proteins. Medicinal plants such as *Cissampelos pareira*, *vitex negundo*, *vetiveria zizanioides*, and *piper nigrum* have effective antiviral properties against this virus.

The alcoholic extract of *Cissampelos pareira* has potent antiviral activity against all the serotypes of dengue viruses. This was evaluated by using the cell-based bioassays as well as plaque reduction assay, assessed for viral replication. Later the invivo study was performed by using cipa extract in the AG129 mouse model (Sood et al., 2015). Then the ethanolic extract from the medicinal plant *vitrex negundo* has an antiviral effect against DENV which was more explicitly effective after the posttreatment condition. It was also found to be that based on the doses the inhibitory effect on the virus also differs and hence the extracts of *Vitrex negundo* have a dose-dependent inhibitory effect against DENV (Alagarasu et al., 2022b). The computational study was also performed by using the medicinal plants *Vetiveria zizanioides* and *piper nigrum* against DENV. The compound Ethyl 4-(4-methylphenyl)-4-pentenoate from the plant *Vetiveria zizanioides* has a strong binding affinity against the NS2B-NS3 protease of all the four serotypes of DENV (Lavanya et al., 2016). Whereas the Piperine compound from the plant *Piper nigrum* significantly binds to the Methyltransferase of DENV. This compound when compared to Ribavirin has more affinity towards the viral protein (Nag & Chowdhury, 2020a).

2.3.4 ZIKA VIRUS

Zika virus is an arthropod-borne virus that belongs to the family *Flaviviridae*. Usually, the Zika virus gets transmitted to the host by a mosquito bite. The fever caused by Zika is mostly unspecific with its symptoms and it is difficult to diagnose and misconceived as Dengue, Chikungunya, or any other infectious disease. This Zika virus is weighed by using the invitro studies by using the ethanol extract from the medicinal plant *Ocimum basilicum*. The extract from this plant hinders the virus at the attachment step and host cell entry. This was evidenced by using the in vitro study in the Vero E6 cell line which has a half inhibitory concentration (IC50) value of 1:134. Even the diluted concentration of the extract along with the ethanol used for the extraction was found to be nontoxic in the cell line (Singh et al., 2019).

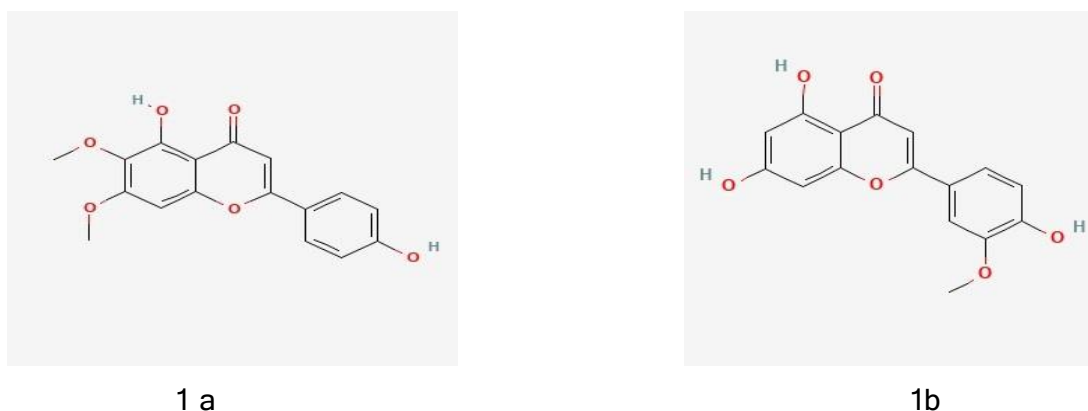
2.3.5 HEPATITIS C VIRUS

The Hepatitis C virus belongs to the family *Flaviviridae* and Genus *Hepacivirus*. This virus is generally transmitted through the blood and causes liver cirrhosis and primary hepatocellular carcinoma (Modrow, Falke, et al., 2013). The compound chebulagic acid extracted from the plant *Terminalia chebula* has been computationally studied by using docking tools. This compound effectively inhibits the HCV NS3/4A protease enzyme present in the Hepatitis C virus (Ajala et al., 2014; Patil et al., 2022). The compounds trans-anethole, estragole, limonene, fenchyl acetate, trans- β -ocimene, and α -pinene extracted from the plant exhibited the unique antiviral property by hindering the viral enzyme namely HCV NS5B polymerase enzyme. This binding capacity of the compounds was analysed by using computational tools (Ibrahim & Moussa, 2021b).

2.3.5 SARS-COV-2

Coleus amboinicus belongs to the *Lamiaceae* family and is traditionally used for treating various lung infections that are caused by pathogens (Ślusarczyk et al., 2021). The plant extracts generally possess phenolic compounds such as carvacrol, flavonoid, rosmarinic, caffeic acid, and chlorogenic acid. The flavonoids are generally composed of large subgroups of polyphenolic compounds (Solnier &

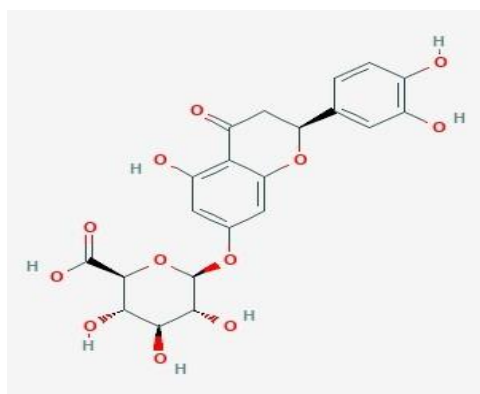
Fladerer, 2021). The flavone Cirsimaritin and chrysoeriol (Fig:1 &2) extracted from this plant had a good binding affinity with the SARS COV-2 spike glycoprotein. These compound binding affinities with the spike glycoprotein have been computationally analyzed by using docking tools. The LF ranking score for the compound Cirsimaritin was -9.228 and for the compound, chrysoeriol was -11.392. The high negative values of the LF ranks describe the high binding affinity property towards protein and ligand binding. Hence the compound Chrysoeriol has a high binding affinity whereas the compound Cirsimaritin has a moderate binding affinity(Kiran et al., 2022b).



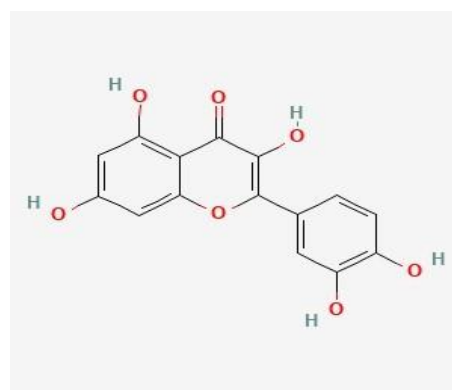
(Figure 2.1: In figure 1a represent the chemical structure of the compound Cirsimaritin (PubChem ID:188323) and 1b represents the chemical structure for the compound Chrysoeriol (PubChem ID:5280666))

The flowering plant Citrus limon belongs to the family Rutaceae and has a valuable potency in food and medicines. It is also a good antioxidant and radical scavenging property. The essential oil extracted from this plant has 21 different components present in it (ben Hsouna et al., 2017). The compounds eriodictoyl, quercetin, and diosmetin have a potential antiviral property against the two SARS COV-2 main protease enzymes namely 3CL^{pro} (chymotrypsin-like protease) also known as M^{pro} and PL^{pro} (papain-like protease). These enzymes are responsible for viral replication and involve in non-structural protein maturation. The computational study was performed with these compounds against the main protease enzymes and determined by using the comparative data with standard antiviral drugs. The results showed that these compounds have potential binding energy when compared to

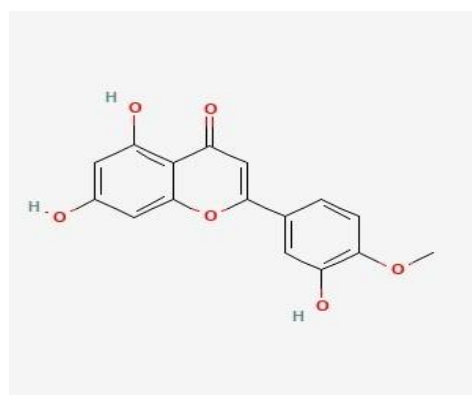
standard antiviral drugs(Khan et al., 2022). The plant *Citrus aurantiifolia* is possessed to have potential antimycobacterial activity against the *Mycobacterium tuberculosis* H37Rv strains (one sensitive and three multidrug-resistant strains). The hexane extract was prepared, and these compounds were screened for antimycobacterial activity by using MABA (Microplate Alamar Blue Assay). The compounds 5,8-Dimethoxypsoralen and palmitic acid with MIC values 25–50 $\mu\text{g/mL}$ have found to be the potential antimycobacterial constituent against all the strains(ben Hsouna et al., 2017).



2a



2b



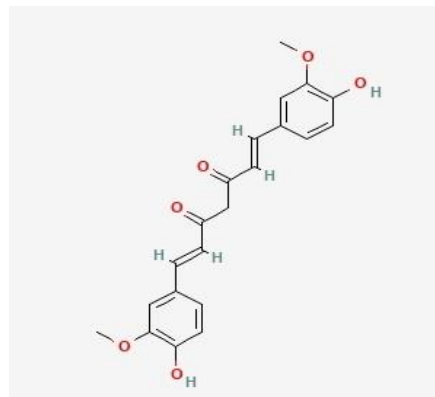
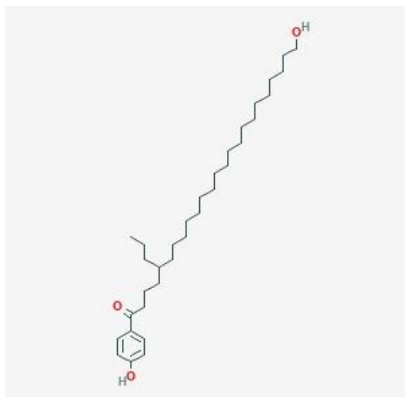
2c

(Figure 2.2: The figure 2a, 2b, and 2c represent the chemical structure for the compound eriodictoyl (PubChem id: 10412099), quercetin (PubChem id: 5280343), and diosmetin (PubChem id: 5281612) respectively).

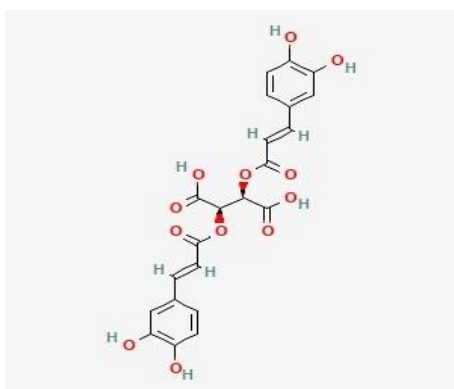
The compound 4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)- phenol extracted from the plant *Leucas aspera* has been studied computationally. The compound was docked against the SARS COV-2 spike protein and the results indicated that this compound has potential binding affinity when compared to antiviral drugs such as Hydroxychloroquine and Remdesivir(Navabshan et al., 2021b).

The medicinal plant *Curcuma longa* compounds such as curcumin prevents the SARS COV -2 viral replication by hindering the 3CL protease in the Vero E6 cell line. The methods used for this study were cell-based CPE assay and SARS-CoV 3CL protease inhibition assay (Wen et al., 2007). The plant *Ocimum gratissimum* is computationally studied against the SARS COV-2 virus by using molecular docking. The 3CL^{pro} (3- Chymotrypsin Like protease enzyme helps the virus in replication inside the host. The compounds chicoric acid, luteolin, and myricetin have a potential binding tendency against this protease enzyme (Gyebi et al., 2021b).

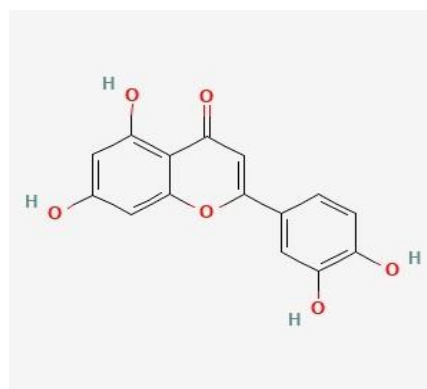
The compounds Agnuside, Friedelin, Luteolin, 7-O-(6''-malonylglucoside, Luteolin 7-O-beta-D-glucoside, Luteolin-7-o-beta-d-glucopyranoside, oleanolic acid, ursolic acid and 3 β -acetoxyolean-12-en-27-oic acid extracted from the plant *Vitex negundo* exhibits the significant binding against the SARS COV-2 proteins namely spike protein (structural protein), NSP3, NSP9, NSP15 (non-structural proteins), and main protease enzyme. The computational study result shows that these compounds have a synergistic interaction between compounds and protein targets (Nallusamy et al., 2021).



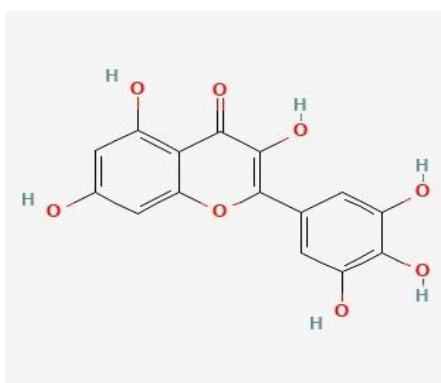
3a



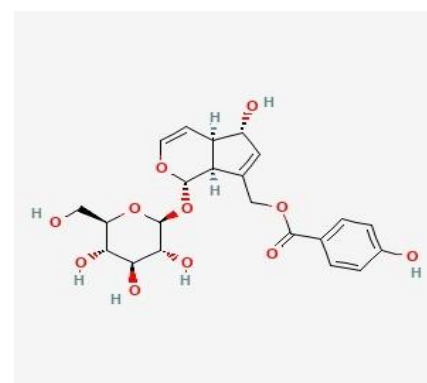
3b



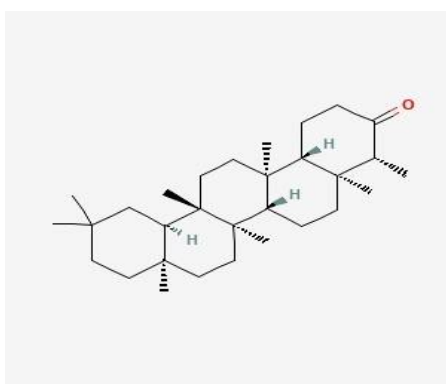
3c



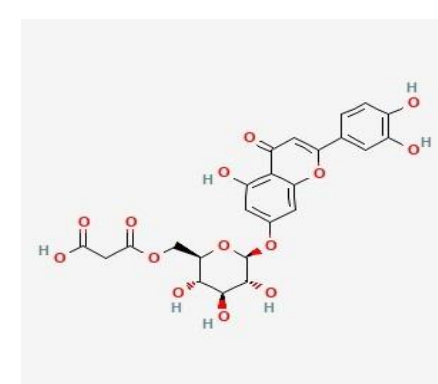
3d



3e



3f

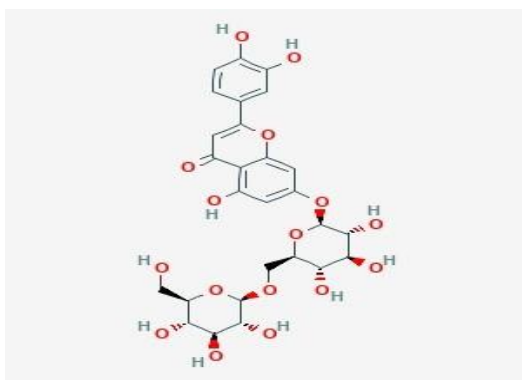


3g

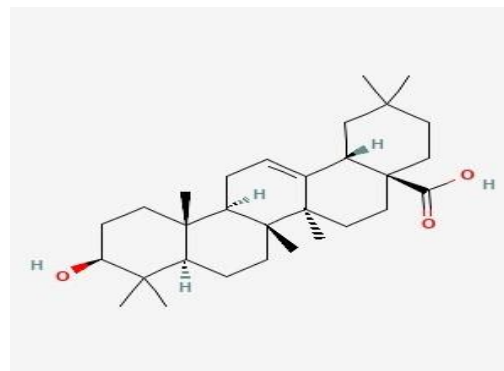


3h

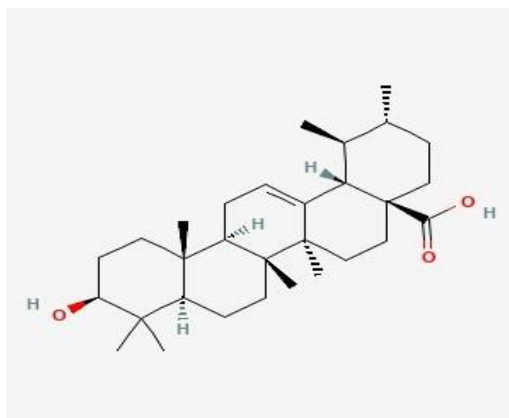




3i



3j



3k

(Figure 2.3: The figure 3a represents the chemical structure of the compound 4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)- phenol (PubChem id: 90802206), 3b represents the compound Curcumin (PubChem id:969516). The figure 3c,3d, and 3e represents the compound chicoric acid (PubChem id: 5281764), luteolin (PubChem id: 5280445), and myricetin (PubChem id: 5281672), and the figure 3f, 3g, 3h, 3i, 3j, and 3k represents the compounds Agnuside, Friedelin, Luteolin, 7-O- (6''-malonylglucoside, Luteolin,7-O-beta-D-glucoside, oleanolic acid, and ursolic acid)

2.4 NEGATIVE SENSE ssRNA VIRUS

The negative sense ssRNA viruses including the Respiratory syncytial virus and influenza virus that has been effectively hindered by the phytocompounds extracted from the medical plants.

2.4.1 RESPIRATORY SYNCYTIAL VIRUS

The plants *Barleria prionitis* and *Mentha piperita* showed effective antiviral properties against Respiratory syncytial virus (RSV) through in vitro studies. By using the Hep2 cells the antiviral activity was performed against RSV by using the compound 6-O-trans-p-coumaroyl-8-O-acetylshanzhiside methyl ester and its cis isomer from the medicinal plant *Barleria prionitis*. This compound inhibits RSV by inhibiting the virus-induced cytopathic effect (Chen et al., 1998b). Also, the ethanolic extract from the plant *Mentha piperita* contains high phenolic and flavonoid compounds that have the potential antiviral property. The compound toxicity was analysed by using the cytotoxicity assay and the compound binding with the virus was analysed by using the Antiviral assay. Since the compounds contain phenolic compounds, they can easily spread to the lungs and have diverse effects on them (Li et al., 2017).

2.4.2 INFLUENZA VIRUS

The Influenza virus contains sialic acid receptors that are responsible for the replication of the virus. The methanolic extract obtained from the plant *Adhatoda vasica* blocks the viral adsorption to the host cells. This mechanism of blocking viral adsorption is analysed by using the post-treatment and a simultaneous assay. These assays conclude that the extracts might hinder the replication of the virus by inhibiting the viral HA protein (Rahul Chavan & Abhay Chowdhary, 2014). The crude and terpenoid extract from the plant *Ocimum sanctum* exhibit significant antiviral properties against the H9N2 strain of the Influenza virus. The antiviral efficacy of these compounds was tested on the Ovo model by using the therapeutic (time-dependent) and prophylactic (dose-dependent) activity assays and real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) assays were carried out to analyze the virulence of the virus after the post-treatment (Ghoke et al., 2018). The computational studies were also carried out in these plant compounds namely Apigenin against the Neuraminidase (NA) and Hemagglutinin (HA) of the H1N1 strain of the influenza virus (Alhazmi, 2015).

The extract from the plant *Cinnamomum cassia* has a significant antiviral property against the H7N3 Influenza A virus. The toxicity of the compound was analysed by

using the invitro cytotoxicity assay and the antiviral property of the plant were tested by using the Infectivity assay (Fatima et al., 2016).

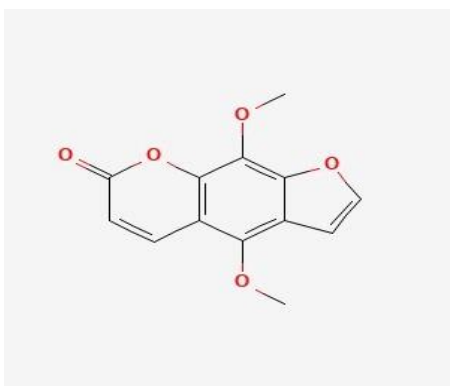
2.4.3 EBOLA VIRUS

The Ebola virus causes hemorrhagic fevers and transmits through the infectious fluids of the infected person. The compound piperine extracted from the plant has more affinity towards the viral protein when compared to the antiviral drug namely Ribavirin. The binding capacity of this compound was studied by using the computational tool, resulting in the inhibition of the VP35 interferon inhibitory domain of this virus(Nag & Chowdhury, 2020b).

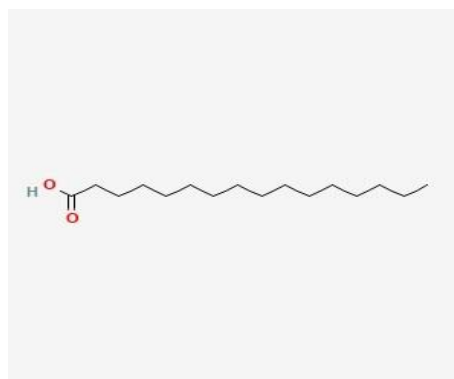
2.5 ANTIMYCOBACTERIAL ANALYSIS AGAINST MYCOBACTERIUM TUBERCULOSIS

The essential oil extracted from the plant *C.amboinicus* is said to have antimycobacterial properties against *Mycobacterium tuberculosis*. The essential oil was tested with the Resazurin Microtiter Assay method (REMA) and determined by using the Minimum Inhibitory concentration (MIC) values and its values were found to be $351.6 \pm 39.06 \mu\text{g/mL}$.(Mota et al., 2018).

The plant *Citrus aurantiifolia* is possessed to have potential antimycobacterial activity against the *Mycobacterium tuberculosis H37Rv* strains (one sensitive and three multidrug-resistant strains). The hexane extract was prepared, and these compounds were screened for antimycobacterial activity by using MABA (Microplate Alamar Blue Assay). The compounds 5,8-Dimethoxypsoralen and palmitic acid with MIC values $25\text{--}50 \mu\text{g/mL}$ have been found to be the potential antimycobacterial constituent against all the strains(ben Hsouna et al., 2017).



4a



4b

(Figure 2.4: The figure 4a and 4b represents the compound 5,8-Dimethoxypsoralen and palmitic acid respectively).

Chapter 3

AIM AND OBJECTIVE

3.1 AIM

To formulate the inhaler or capsule against lung infections from medicinal plants.

3.2 OBJECTIVE

- To identify the compounds, present in the plant extract by using GCMS
- To screen the compound interacting with SARS COV -2 and Mycobacterium tuberculosis H37rv protein targets using *INSILICO* computational analysis.
- To analyse the screened compounds by using *INVITRO* studies (cytotoxicity and protein binding assays)
- To prepare cell stock by using *Mycobacterium tuberculosis H37rv*.
- To determine the extract susceptibility quantitatively by using Microplate Alamar Blue Assay (MABA)
- To analyse the statistical Minimum Inhibitory Concentration (MIC) Values calculated from the arithmetic mean of the sextuplicates on the plate.

Chapter 4

4. MATERIALS AND METHODS

4.1 Sample collection and Preparation

The medicinal plants were screened based on their medicinal value properties from the literature survey. *Coleus amboinicus*, *Citrus limon*, *Leucas aspera*, *Curcuma longa*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum gratissimum*, *Vitrex negundo*, and *Allium sativum* were grown and leaf parts were collected, and shade dried under normal room temperature. The dried sample was ground into powder and stored for further use.

4.2 Extraction

Initially, compounds are removed from the plants by adding suitable solvents to the extraction. The polar and non-polar solvents were selected based on their purposes. Here, the extraction was performed by using the aqueous solvent in the Soxhlet apparatus.

The 35g-45g of leaves from the plant *Coleus amboinicus* leaves were weighed individually and taken as sample 1(S1). In addition to that, the leaves of the plant *Coleus amboinicus*, *Citrus limon*, *Leucas aspera*, *Mentha piperita*, *Ocimum basilicum*, *Ocimum gratissimum*, and *Vitrex negundo* were weighed around 6g each and the rhizome parts of the plant *Allium sativum*, and *Curcuma longa* were weighed around 3g each and labelled as a Sample 2. Now Sample 1 and Sample 2 were individually subjected to the Soxhlet extraction by adding 250-500 ml of the solvent (Aqueous) to the Soxhlet. The Soxhlet was made to run around 6-8 cycles completely. Then the extract obtained from both samples was cooled and stored at 4°C.

4.3 Rotary evaporation

Rotary evaporation is performed to concentrate the sample by the removal of the solvents present in it. The extract obtained from the Soxhlet was subjected to this

process and obtained as crude. For sample 1, 2.7g of crude was obtained and for sample 2, 3.7g of crude was obtained.

4.4 Phytochemical screening

The plant extracts contain secondary metabolites which are released by the plants. These secondary metabolites can be identified by using respective phytochemical screening tests. The tests include the identification of Saponins, Tannins, Phlobatannin, Flavonoids, Steroids, Terpenoids, Cardiac Glycosides, Phenols, Quinones, and Alkaloids

4.4.1 Test for Saponins

2ml of distilled water and 2ml of the extract is taken in a graduated cylinder and vigorously shaken for 15 minutes. The formation of a 1cm layer of foam indicates the presence of Saponins.

4.4.2 Test for Tannins

1ml of 5% Ferric chloride (FeCl_3) solution is added with 1ml of extract. The presence of dark blue or greenish-black colour indicated the presence of Tannins.

4.4.3 Test for Phlobatannin

1ml of extract was boiled with 1ml of 1% Hydrochloric acid. The deposition of red precipitate in the test tube indicates the presence of Phlobatannin.

4.4.4 Test for Flavonoids

1 ml of the extract was mixed with a few drops of dilute sodium hydroxide solution. on adding the dilute acid to that mixture, the yellow colour gets disappeared which indicates the presence of Flavonoids.

4.4.5 Test for Steroids

2ml of chloroform and 1ml of concentrated sulphuric acid were mixed with the 0.5ml of extract. The formation of a reddish-brown ring at the interface of the test tube indicates the presence of Steroids.

4.4.6 Test for Terpenoids

0.5ml of the extract was mixed with 2ml of chloroform and a few drops of concentrated sulphuric acid. The formation of reddish-brown colour at the interface indicates the presence of Terpenoids.

4.4.7 Test for Cardiac Glycosides

2ml of extract was added with 0.5ml of glacial acetic acid, concentrated sulphuric acid, and two to three drops of ferric chloride solution in the test tube. The appearance of deep blue colour at the junction indicates the presence of Cardiac Glycosides

4.4.8 Test for Phenols

To the 1ml of extract 2ml of distilled water and 10% Ferric chloride solution were added to the test tube. The formation of blue or green colour indicates the presence of phenols.

4.4.9 Test for Quinones

1 ml of the extract was mixed with 1 ml of concentrated sulphuric acid. The appearance of red colour indicates the presence of Quinones.

4.4.10 Test for Alkaloids

To the 2ml of the extract 1ml dilute hydrochloric acid and 1ml of Wagner's reagent were added to the test tube. The presence of a reddish-brown precipitate indicates the presence of Alkaloids.

4.5 Antioxidant assay

The antioxidant activity of the plant is analysed by using the DPPH (Diphenyl-1-picrylhydrazyl) radical scavenging activity. The free radical scavenging capacity of the plant extract was measured based on the method delineate by Brand-Williams *et al* (1995) with slight modification. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (50, 100, 150, 200 and 250 µg/ml). Blank samples were prepared, and L-Ascorbic acid was used as standard. These tubes were thoroughly mixed and kept in the dark for 30 min and their absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c is the absorbance of the control

A_s is the absorbance of the sample

4.6 Haemolytic assay

The activity of the plant extracts over the human erythrocytes was calculated by using this haemolytic assay. For performing this assay goat blood was collected in the EDTA tubes. 1ml of blood was centrifuged to remove the plasma layer then PBS wash was given three times. Now the pelleted erythrocytes were mixed with basic saline. From that 50µl of the blood is taken in the tube and 100µl of the sample in different concentrations (25µg/ml, 50 µg/ml, 100 µg/ml, 150 µg/ml, 200 µg/ml, 250 µg/ml, 500 µg/ml) was taken. 0.1% Triton x100 as a positive control and 1X PBS as a negative control. Then the tubes were incubated for one hour at 37 °c. After incubation 850µl of the 1X PBS was added to each tube and centrifuged at 3000rpm for 3min. the supernatant obtained from the centrifugation was transferred into the 96microtiter well plate and observed under 540nm.

$$\text{Percentage of Hemolytic activity} = \frac{\text{ABS of Sample} - \text{ABS of Negative control}}{\text{ABS of Positive control} - \text{ABS of Negative control}} \times 100$$

4.7 Chromatographic techniques

4.7.1 GCMS

GC-MS is chromatographic method that helps to identification and quantification of secondary metabolites in each plant extract. GC-MS is the relatively high-coverage method used for the identification of compounds in a single run. GC-MS can able to perform two functions such as individually and concomitantly. it compares the relative concentrations with atomic masses. More data can be observed and simultaneously correlated by computers for the identification of values from the raw GC-MS data. The original method of analysis helps to measure the ranges of a peak. The identification of compounds which is present in the given sample is automatically collected by programs. GC-MS is a fast and highly efficient analysing method for identifying compounds.

4.7.2 Column chromatography

Column chromatography is used to separate the single compound from the crude by using the single solvent system. The column is packed by mixing the silica gel 100-200 mesh with any non-polar solvent (hexane) and gradually pouring it into the column and allowing the silica to get settled without any air bubbles. Once the column is set, initially the solvent which is used to pack the column is poured and allowed to run completely down the column. After this, the sample coated with the silica gel 60-120 mesh is subjected to the column and allowed to settle inside it. The column should never get dried, this makes the packing leave some air bubbles in it.

For eluting the compound from the mixture, the solvents should be used from non-polar to polar solvents. Individually each solvent is allowed to run completely and the elutes are collected in separate test tubes. After this for washing the column acetone is added and allow the column runs completely.

4.7.3 TLC

To analyse the wide range of biomolecules from the plants at a greater speed and efficiency this technique is used. The TLC plates are made into thin sheets coated with silica slurry which has the tendency to separate the biomolecules. Here, the stationary phase is taken as a sample, and the mixture of solvents is taken as a

mobile phase. The mobile phase solvents are made it into ration based on their polarity.

Draw a line 1.5-2.0cm from the top and bottom of the silica plate and place the marking to spot the sample. Each spot should be placed in 0.5cm of the gap. Now take the capillary tube and place it in the spotted area. Place the plate in a 45- degree angle inside the beaker containing the required solvent system (mobile phase). Allow the solvent to travel until it reaches the marked line at the top and observe the spots or bands under the UV illumination. Measure the RF value by using the formula.

$$\text{Rf value} = \text{Distance travelled by solute} / \text{Distance travelled by solvent}$$

4.8 Computational analysis

4.8.1 Toxicity identification

Toxicity identification is a method used to predict the toxicity of the compounds in the plant sample. Identification of toxicity is performed using any tools by *insilico* analysis is called as in silico toxicology (IST). Toxicity can be determined based on the molecular structure of compounds and their chemical properties. Uses of this prediction is to find out the toxic chemicals easily and can be performed at any time as required for drug development. Osiris property explorer software is a tool used to predict the toxicity level of compounds. Collect the drug-related properties when the structure is valid. It shows two colours such as red and green colour. The red colour shows the getting high risk of effects and the green colour indicates the good behavioral properties of the drug. It requires a short time to analyse the compounds and interpret the data.

4.8.2 ADMET Profiling

Admet profiling is a major part that plays in the drug-designing process. Admet properties can cause 50-60% of all drugs be failed at the clinical phase of drug development. Lack of safety and drug efficacy are the major properties to cause drug failure. Admet profiling was performed by *insilico* analysis using the

Swissadme tool. In this tool, the drug-likeness and drug score were calculated based on Lipinski's rule. This rule includes.

4.8.3 Molecular docking

Molecular docking is a computational technique that is used to study the interaction of small molecules (ligands) with the target binding site (protein). This technique is widely used for structure-based drug design to repurpose many formulations. The tools used for molecular docking are Autodock, patch dock and Discovery studio etc. the

4.9 Antibacterial assay

The antibacterial assay is used to find the inhibition property of the extract against various bacterial species by using the well diffusion method. Here the bacterial strains namely *E. coli*, *Bacillus cereus*, and *Staphylococcus aureus* were used for an experiment. The overgrown culture of these strains was initially inoculated in the LB broth and allowed to grow overnight. The Mueller- Hinton agar plates were prepared, and these cultures were patched over the surface of the agar by using cotton swabs. Then the wells are created by using the puncher and each well was loaded with 100µl of samples in different concentration. The antibiotics were taken as a positive control and incubated the plates at 37 °c overnight. Hence, based on the zone of inhibition the antimicrobial activity of the plants was calculated.

4.10 Antimycobacterial assay

The antimycobacterial assay is performed to find the inhibition property of the extract against the mycobacterial strains by using the well diffusion method. The strain *Mycobacterium smegmatis* is cultivated in the Middlebrook 7H9 broth and incubated overnight for their growth. After incubation prepare the 7H11 agar plates and patch the grown cultures over the surface of the agar by using the cotton swabs. Then the wells were punched by using the puncher and each well was loaded with 100µl of samples in different concentration. The antibiotics were taken as a positive control and incubated the plates at 37 °c overnight. If the extracts and crude

samples inhibit the growth of the mycobacterium strain the zone of inhibition will be formed.

4.11 MABA Assay

The MABA assay defines the high throughput screening of the drugs by using the specific reagent Alamar blue which can be detected calorimetrically by the change in colour. This assay was performed by using the flat bottom 96 well plates. The 100µl of the fresh Middlebrook 7H9 broth is added with 100 µl of the Mycobacterium smegmatis culture (overnight culture). Then 100 µl of different concentrations of the sample 1 and 2 (100 µg/ml, 200 µg/ml, 400 µg/ml, 600µg/ml, 800 µg/ml, 1000 µg/ml) were added to each well, and kanamycin was taken as a positive control in a single well. Then the whole setup is incubated for 48 hours and after that 35 µl Alamar blue dye mixed with 1X PBS was mixed with 20% Tween 80 in an 8:5 ratio was added to each well. Again, the whole setup is incubated for 24 hours at 37 °C. The change in colour of the plate from blue to pink indicates the growth of mycobacteria and no colour change indicates a positive result.

4.12 Cytotoxicity- MTT assay

The MTT assay is used to predict the cytotoxicity of the obtained extract and crude. The crude and aqueous extracts of sample 1 and 2 were used to analyse the cytotoxicity. HEK293T (Human embryonic kidney cell line) was grown in a suitable medium and checked for its growth viability. Then the cells were counted in the hemocytometer such that when the cells were added in each well, it would contain 5000 cells per 100µl. Once the cells are seeded along with equal amount of fresh media, it is incubated for 24hrs. The 100µl of drug with different concentrations were added to each well. Then the positive control is taken as Triton X 100 and incubated for 24hrs. After this media is discarded and 100µl of PBS and 10% of 10µl of MTT is added and incubated it for 2 hours. Then the 100µl of solubilization solution were added and kept in the shaker for 1 hour to solubilize the crystals formed by MTT solution and observed the reading under 570nm.

Chapter 5

RESULT AND DISCUSSION

5.1 Extraction of phytomolecules

The selected plants were extracted by using the Soxhlet extraction. The plant leaves were washed thoroughly and shade-dried and extracted by using the aqueous solvent. Then the extract is sent for rotary evaporation and the crudes are obtained from each sample.

Sample number	Collected sample	Amount of samples taken	Amount of solvent used (Aqueous)	Amount of crude obtained
1	<i>Coleus amboinicus</i>	35g	250ml	2.7g
2	<i>Coleus amboinicus</i>	6g	400ml	3.7g
	<i>Citrus limon</i>	6g		
	<i>Leucas aspera</i>	6g		
	<i>Mentha piperita</i>	6g		
	<i>Ocimum basilicum</i>	6g		
	<i>Ocimum gratissimum</i>	6g		
	<i>Vitex negundo</i>	6g		
	<i>Allium sativum</i>	3g		
	<i>Curcuma longa</i>	3g		

Table 5.1: The table represents the data for extract and crude obtained.



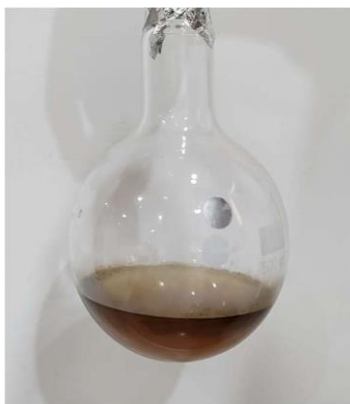
(a)



(b)



(c)



(d)



(e)

Figure 5.1: This figure represents the stages for obtaining the crude extracts

- (a) Drying of the plant
- (b) Packing the dried plant in the filter paper for Soxhlet extraction
- (c) Soxhlet extraction
- (d) Extract obtained from the extraction process.
- (e) Obtained crude from the rotary evaporator.

4.13 Phytochemical screening

To the extract obtained from the extraction the Phyto screening has been performed for both Sample 1 and Sample 2

Label	Phytochemical test	Result for sample 1	Result for sample 2
a	Saponins	Present	Present
b	Alkaloids	Present	Present
c	Terpenoids	Absent	Absent
d	Quinones	Present	Present
e	Cardiac glycosides	Absent	Absent
f	Flavonoids	Present	Present
g	Phenols	Present	Present
h	Tannins	Present	Present
i	Steroids	Present	Present
j	Phlobatanin	Absent	Absent

Table 5.2: This table represents the phytochemical test result for the sample 1 and sample 2

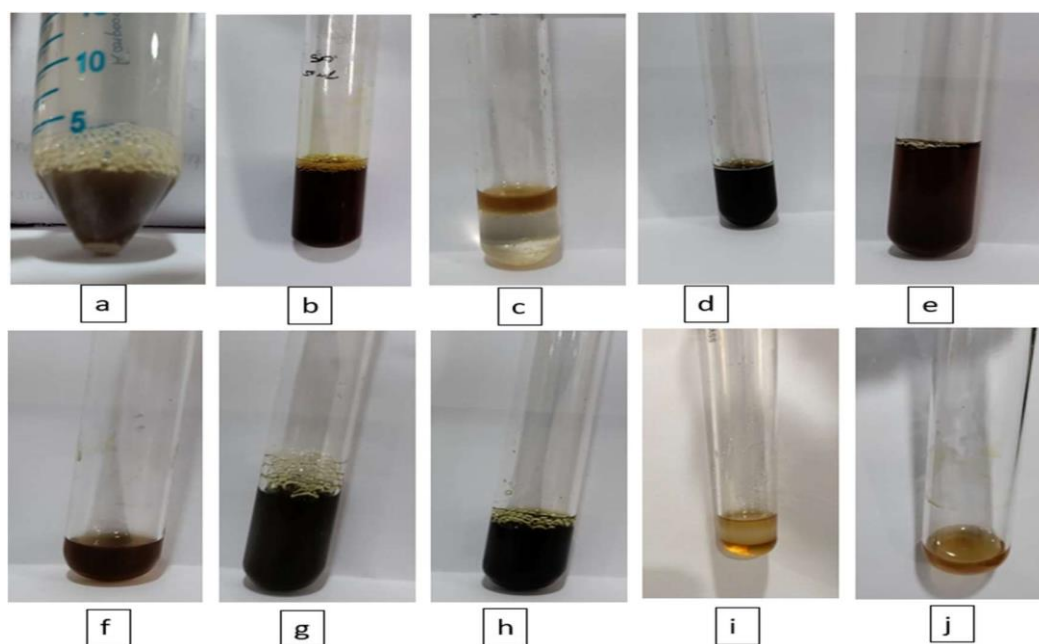


Figure 5.2: The following figure represents the phytochemical screening for sample 1 (*Coleus amboinicus*)

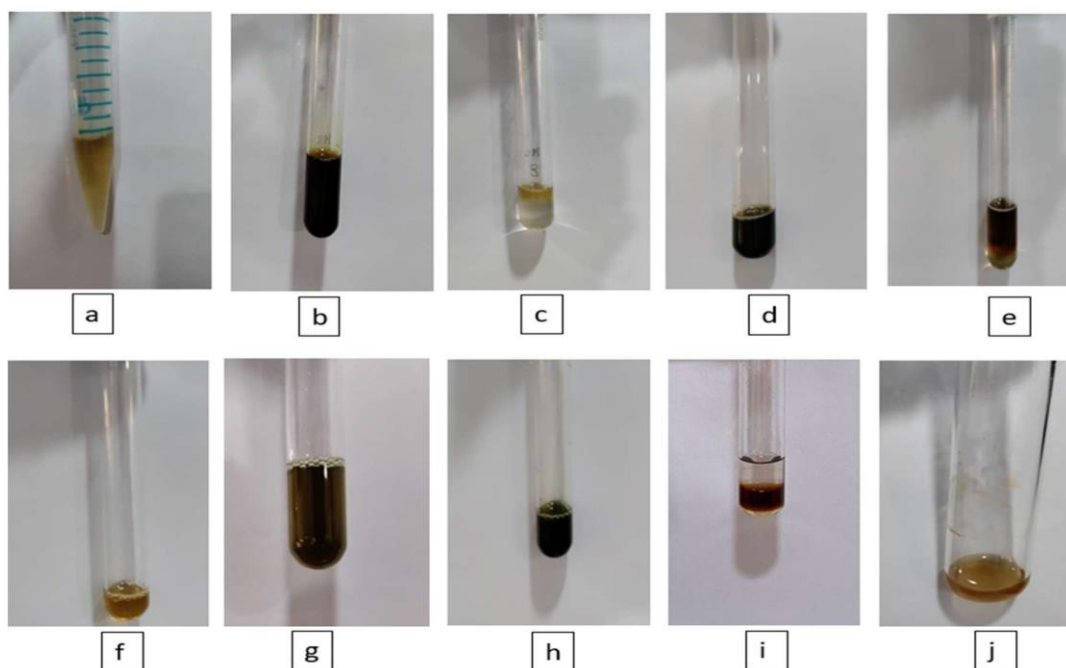


Figure 5.3: The following figure represents the phytochemical screening for sample 2 (9 plant extract)

4.14 Antioxidant assay

The extract and crude of samples 1 and 2 were taken in different concentrations. The 0.1mM DPPH solution is prepared in the ethanolic solution and stored it under dark. The samples in the different concentrations were prepared with the DMSO solutions. To the 40 μ l of sample 2.96 μ l of DPPH solution is added and incubated at room temperature under dark for 30 minutes and DPPH solution was taken as a control. After incubation, the reading was observed under 517nm.

Sample	Concentration	Absorbance at 517nm	% inhibition (%)
Sample 1 Crude	25µg/ml	0.559	73.5
	50 µg/ml	0.568	73.0
	100 µg/ml	0.561	73.4
	150 µg/ml	0.558	73.5
	200 µg/ml	0.573	72.8
	250 µg/ml	0.589	72.0
	500 µg/ml	0.586	72.0
Sample 1 extract	5µl	0.273	87
	10 µl	0.141	93
	20 µl	0.153	92.7
	30 µl	0.190	90.9
	40 µl	0.345	83.6
Sample 2 Crude	25 µg/ml	0.592	71.9
	50 µg/ml	0.584	72.3
	100 µg/ml	0.590	72.0
	150 µg/ml	0.582	72.0
	200 µg/ml	0.930	55.9
	250 µg/ml	0.630	70.1
	500 µg/ml	0.581	72.4
Sample 2 Extract	5 µl	0.199	90.5
	10 µl	0.141	93.51
	20 µl	0.148	92.9
	30 µl	0.227	89.2
	40 µl	0.207	90.1

Table 5.3: This table represents the antioxidant percentage inhibition for both samples. The extracts obtained from both samples have a high percentage of percentage inhibition of free radicals when compared to the crude extracts.

4.15 Haemolytic assay

The haemolytic assay is used to evaluate the erythrocyte lysing property of the sample. Both samples 1 and 2 were used for this assay. haemolytic activity should not exceed more than 10%. When compared to crude the extracts show more percentage of haemolytic activity.

S.No	Sample name	Concentration	+ve Control	-ve Control	OD AT 540nm	Haemolytic activity
1	<i>Coleus amboinicus</i> Crude	25µg/ml	1.588	0.054	0.058	0.26%
		50 µg/ml			0.064	0.65%
		100 µg/ml			0.057	0.19%
		150 µg/ml			0.059	0.32%
		200 µg/ml			0.074	1.30%
		250 µg/ml			0.058	0.26%
		500 µg/ml			0.066	0.78%
2	9 Plant Crude	25µg/ml	1.588	0.054	0.054	0
		50 µg/ml			0.064	0.65%
		100 µg/ml			0.059	0.32%
		150 µg/ml			0.06	0.21%
		200 µg/ml			0.061	0.21%
		250 µg/ml			0.071	0.24%
		500 µg/ml			0.055	0.06%
3	<i>Coleus amboinicus</i> extract	20µl	1.588	0.054	0.1	2.99%
		40 µl			0.165	7.23%
		60 µl			0.185	8.53%
		80 µl			0.22	10.82%
		100 µl			0.132	5.087%
4	9 Plant extract	20 µl	1.588	0.054	0.124	4.56%
		40 µl			0.188	8.73%
		60 µl			0.258	13.2%
		80 µl			0.314	16.9%
		100 µl			0.986	60.7%

Table 5.4: This table represents the haemolytic activity of both the samples.

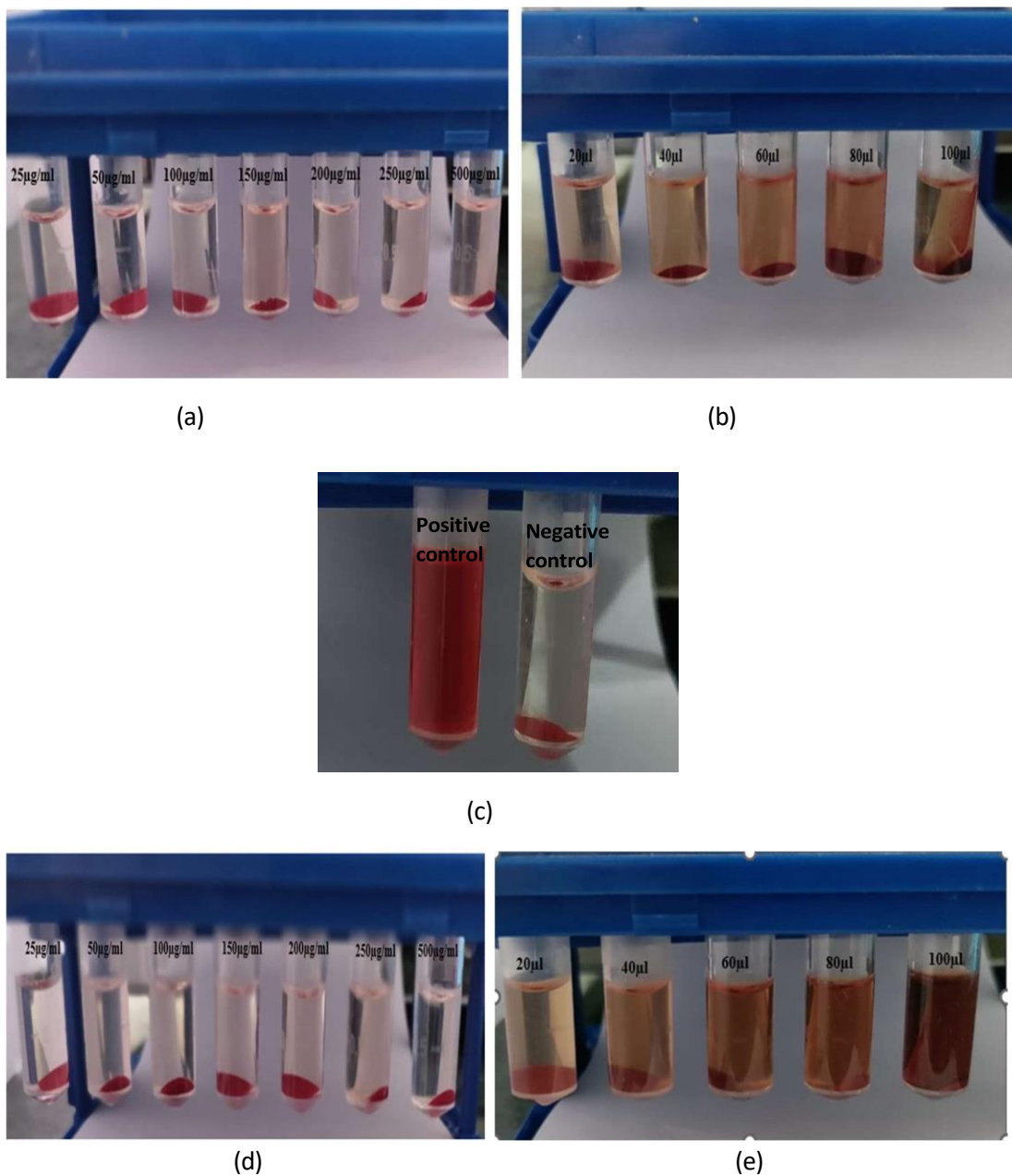


Figure 5.4: This figure represents the haemolytic activity performed for two samples in different concentrations. Here

- (a) *Coleus amboinicus* crude
- (b) *Coleus amboinicus* extract
- (c) Positive and negative control
- (d) 9 plant crude
- (e) 9 plant extract

4.16 Chromatographic techniques

4.16.1 GCMS Report

The following diagrams shows the GCMS report for both the samples.

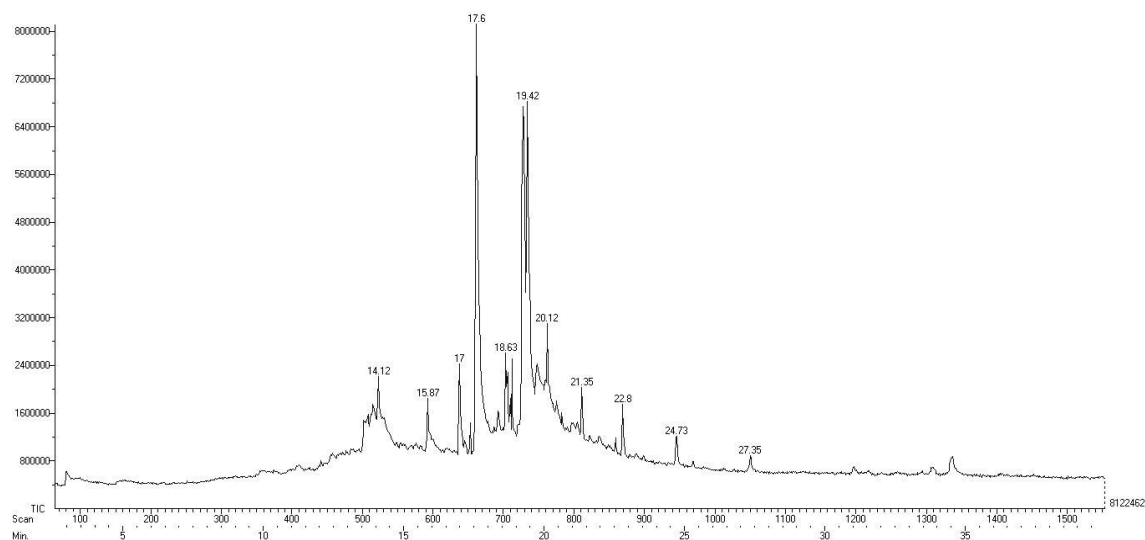


Figure 5.5: This figure represents the peak obtained for the plant extract *Coleus amboinicus* (sample 1)

S.No	Compound names
1	Flavone
2	Dodecanoic acid,11-oxo-methyl ester
3	Cyclododecanecarboxylic acid, methyl ester
4	n- Hexadecanoic acid
5	Oleic acid
6	Octadec-9-enoic acid
7	2H-Naphtalen-1-one, 3,4-dihydro-6-methoxy-2-(4-methoxybenzylideno)
8	Z-13-octadecen-1yl acetate
9	Isopropyl stearate
10	Curan-17-oic acid,19,20-didehydro,-14-hydroxy-methyl ester
11	Pyridine-3-carbonitrile,1,2-dihydro-4-(3,4-dimethoxyphenyl)-6-(2-thienyl)-2-thioxo

Table 5.5: This table represents the list of compounds obtained for the sample 1 from the GCMS report.

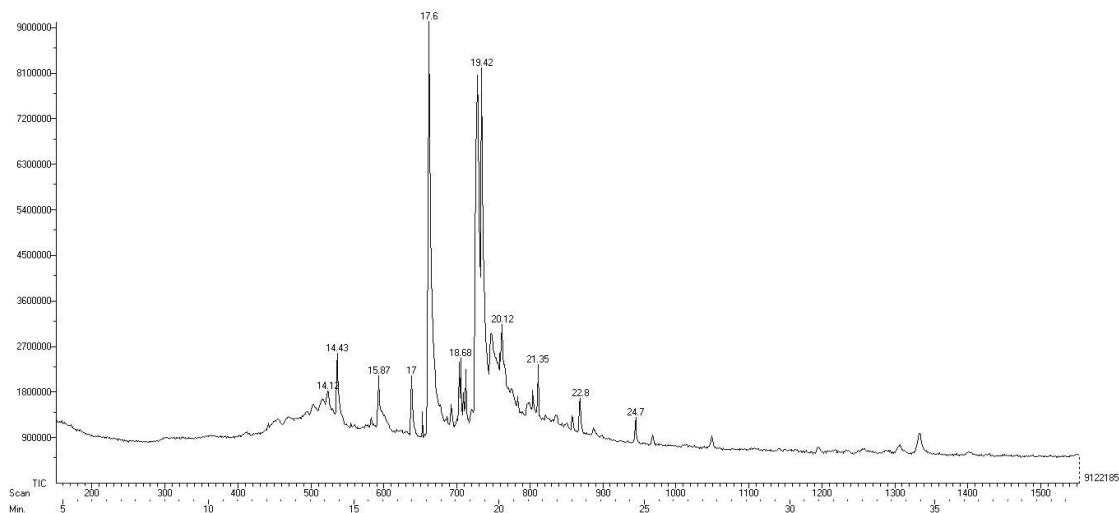


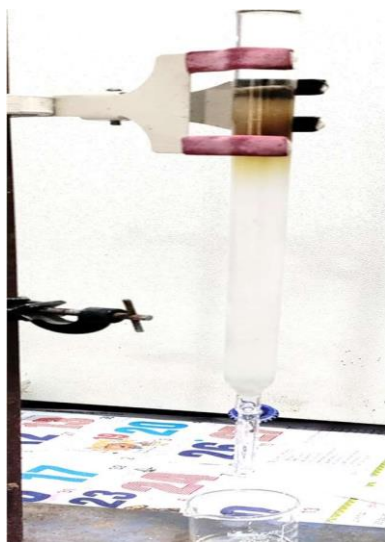
Figure 5.6: This figure represents the peak obtained for the 9-plant extract (sample 2)

S.NO	COMPOUND NAMES
1	1,4,7,10-tetraox-13,15-diazacycloheptadecane-14thione
2	4' methoxy-5,7-dihydroxy flavone
3	7-ethylidenebicyclo(4,2,1)nona2,4-diene
4	n-Hexadecanoic acid
5	Oleic acid
6	Ethanone,1-(2,5 diphenyl 2H-1,2,3-triazol-4-yl 3-oxide)oxime
7	Phenol,2,6-bis(1,1-dimethyl ethyl)-4-[(4-hydroxy-3,5-dimethyl phenyl)]
8	3-methyl-3H-naphth[1,2-e]indol-10-ol
9	Isopropyl stearate
10	Methyl Z-11-tetradecenoate
11	Z-13-octadecen-1yl acetate

Table 5.6: This table represents the list of compounds obtained for the sample 2 from the GCMS report.

4.16.2 Column chromatography

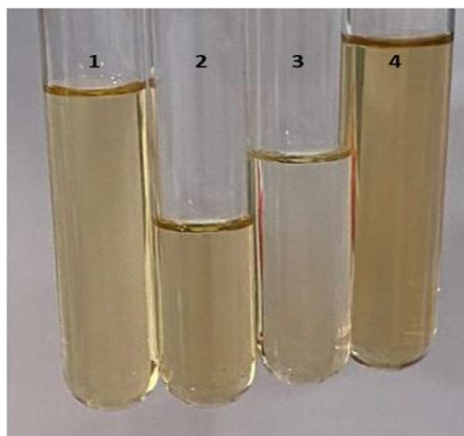
The crude sample were subjected into the column and different elutes were collected. The elute which get eluted from the solvents are n-Butanol, Acetic acid, Methanol, and water.



(a)



(b)



(c)

Figure 5.7: This figure represents the column chromatography performed for the *Coleus amboinicus* (Sample 1)

- (a) Packed column loaded with the sample.
- (b) Crude sample coated with 60-120 mesh grade silica powder.
- (c) Eluted samples from the column are.

- 1- n-Butanol elute.
- 2- Acetic acid elute.
- 3- Methanol elute.
- 4- Water elute.

4.16.3 TLC

The plant *Coleus amboinicus* crude extracts were collected and used for TLC.

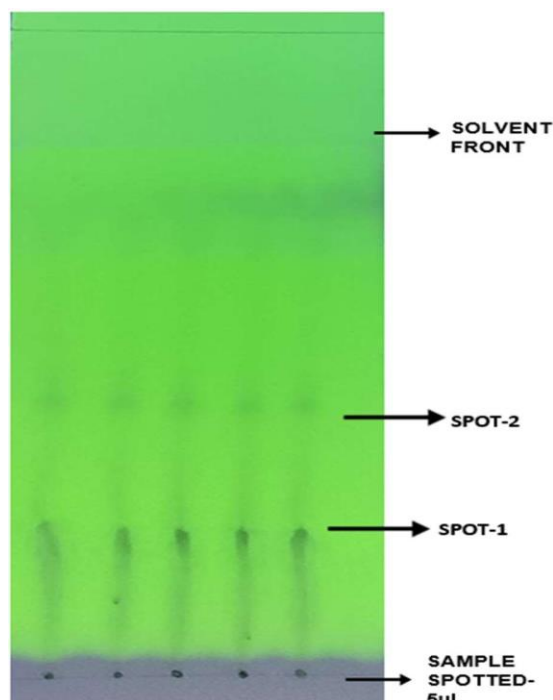
Three spots were formed in two different ratios and their R_f value were calculated and as mentioned below.

Composition	volume	Number of spots	RF value
N-butanol: Glacial Acetic acid: Water	4:1:3	2	0.28,0.49
N-butanol: Methanol: water	4:1:3	1	0.34

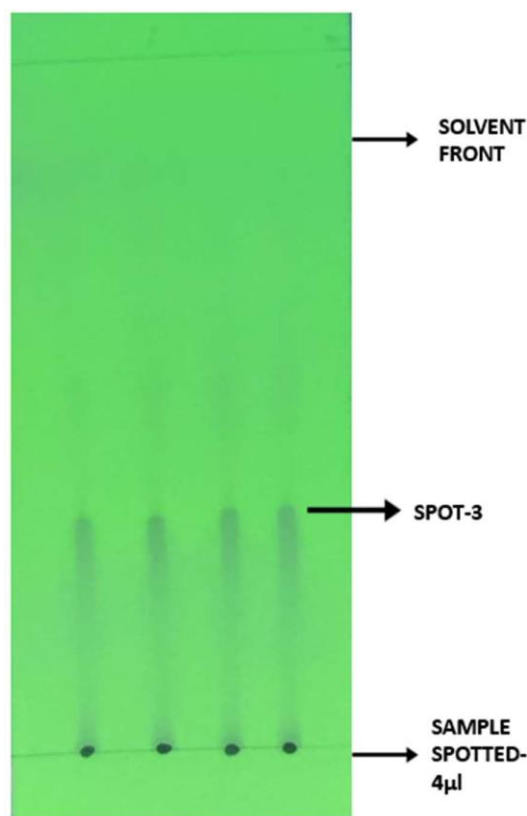
Table 5.7: This table represents the solvent composition and their RF values



(a)



(b)



(C)

Figure 5.8: This figure represents the TLC performed for the sample 1

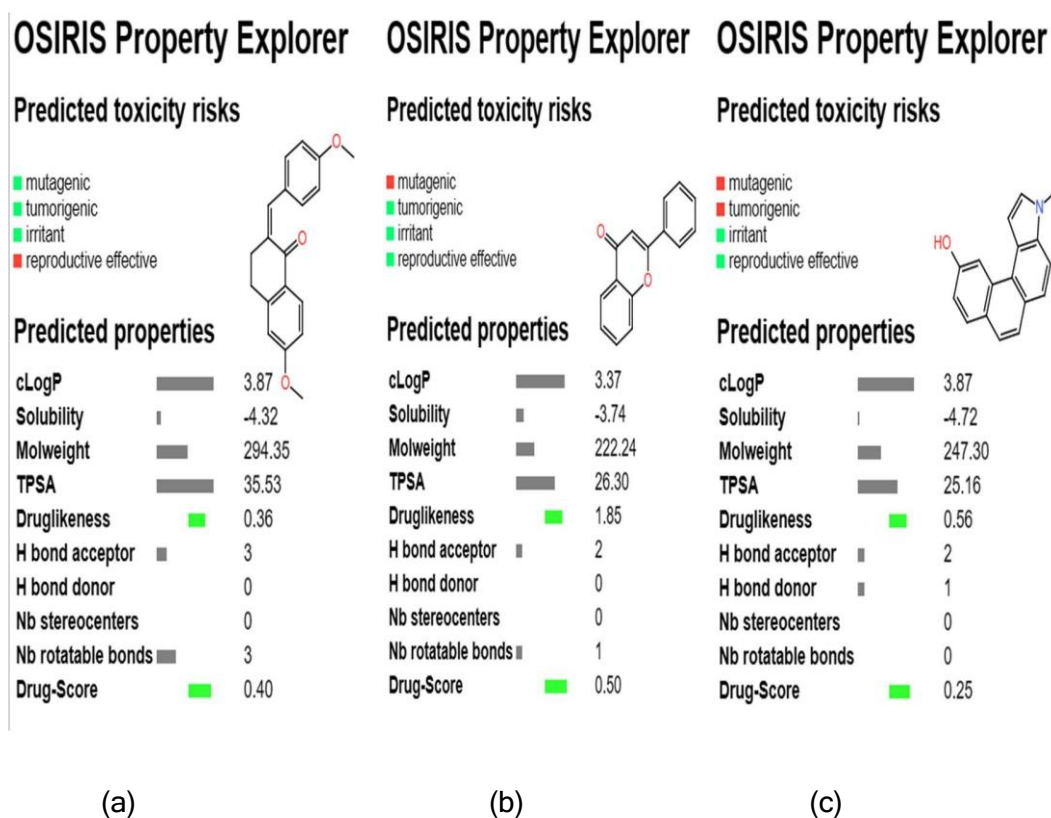
- (a) Sample running in the TLC plate by capillary movement.
- (b) TLC Plate result 1 for the ratio n-butanol: Glacial Acetic acid: Water with two spots
- (c) TLC Plate result 1 for the ratio n-butanol: Methanol: Water with one spots

4.17 Computational study

4.17.1 Toxicity analysis

In silico analysis, Osiris property explorer software was used to predict the toxicity of compounds that are present in the plant of *Coleus amboinicus* and 9 Plants. In this study we analysed that out of 11 compounds from sample1 seven compound namely, Curan-17-oicacid,19,20-didehydro,-14-hydroxy-methylester, Cyclododecanecarboxylic acid, methyl ester, Dodecanoic acid,11-oxo-methyl ester, Isopropyl stearate, Octadec-9-enoic acid, Oleic acid, and Z-13-octadecen-

1yl acetate, passed the toxicity analysis. whereas for the sample 2 out of 11 compounds 8 compounds namely 1,4,7,10-tetraox-13,15-diazacycloheptadecane-14thione, 4' methoxy-5,7-dihydroxy flavone, 7-ethylidenebicyclo(4,2,1)nona2,4-diene, Methyl Z-11-tetradecenoate, Isopropyl stearate, Oleic acid, Phenol,2,6-bis(1,1-dimethyl ethyl)-4-[(4-hydroxy-3,5-dimethyl phenyl)], and Z-13-octadecen-1yl acetate was selected in the toxicity analysis



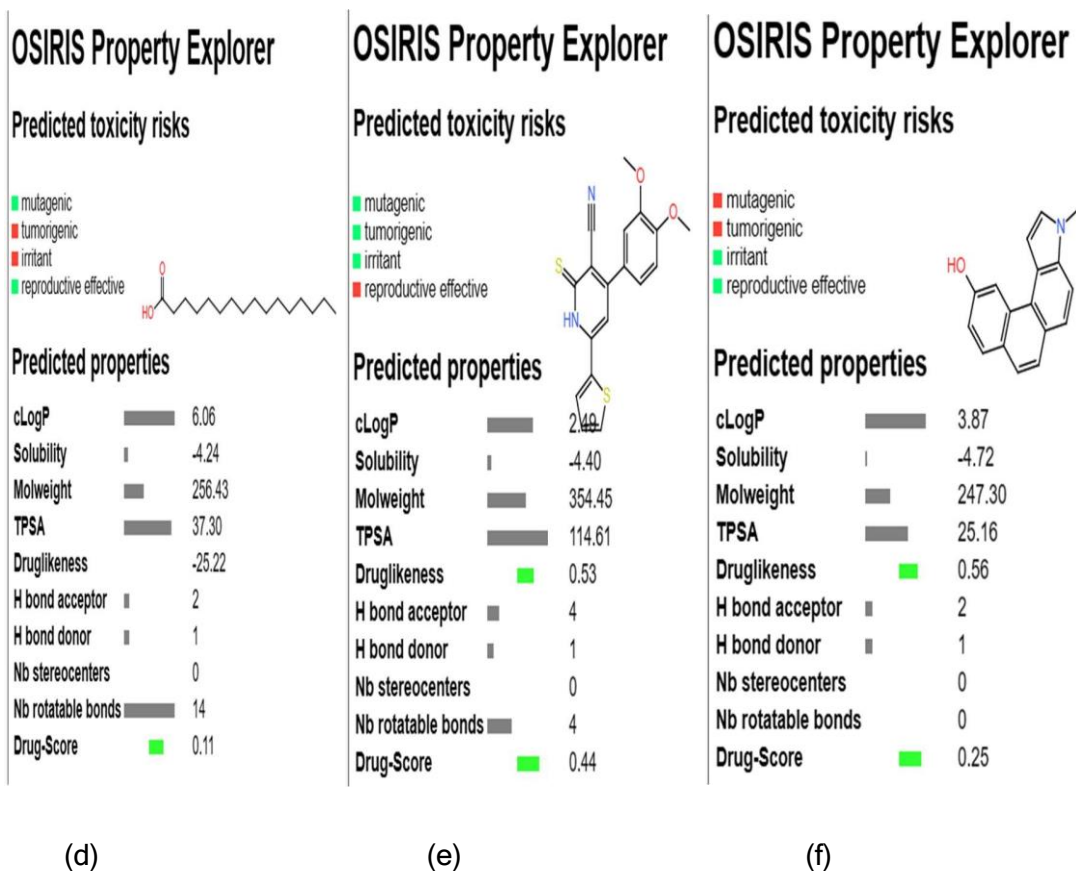


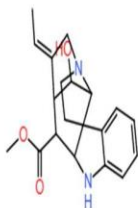
Figure 5.9: This figure represents the toxic compound results obtained from the OSIRIS software.

- (a) 2H-Naphtalen-1-one, 3,4-dihydro-6-methoxy-2-(4-methoxybenzylideno)
- (b) Flavone
- (c) Methyl Z-11-tetradecenoate
- (d) n- Hexadecanoic acid
- (e) Pyridine-3-carbonitrile, 1,2-dihydro-4-(3,4-dimethoxyphenyl)-6-(2-thienyl)-2-thio
- (f) 3-methyl-3H-naphth[1,2-e]indol-10-ol

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

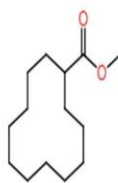
cLogP	1.15
Solubility	-2.84
Molweight	340.42
TPSA	61.80
Druglikeness	1.30
H bond acceptor	5
H bond donor	2
Nb stereocenters	6
Nb rotatable bonds	2
Drug-Score	0.83

(1)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

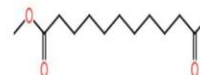
cLogP	4.82
Solubility	-3.53
Molweight	226.36
TPSA	26.30
Druglikeness	-7.78
H bond acceptor	2
H bond donor	0
Nb stereocenters	0
Nb rotatable bonds	2
Drug-Score	0.38

(2)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	3.62
Solubility	-2.94
Molweight	228.33
TPSA	43.37
Druglikeness	-26.54
H bond acceptor	3
H bond donor	0
Nb stereocenters	0
Nb rotatable bonds	11
Drug-Score	0.26

(3)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	8.16
Solubility	-5.59
Molweight	326.56
TPSA	26.30
Druglikeness	-29.04
H bond acceptor	2
H bond donor	0
Nb stereocenters	0
Nb rotatable bonds	18
Drug-Score	0.25

(4)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	6.72
Solubility	-4.55
Molweight	282.47
TPSA	37.30
Druglikeness	-28.97
H bond acceptor	2
H bond donor	1
Nb stereocenters	0
Nb rotatable bonds	15
Drug-Score	0.28

(5)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	6.72
Solubility	-4.55
Molweight	282.47
TPSA	37.30
Druglikeness	-28.97
H bond acceptor	2
H bond donor	1
Nb stereocenters	0
Nb rotatable bonds	15
Drug-Score	0.28

(6)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

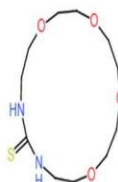
cLogP	7.55
Solubility	-4.98
Molweight	310.52
TPSA	26.30
Druglikeness	-18.81
H bond acceptor	2
H bond donor	0
Nb stereocenters	0
Nb rotatable bonds	17
Drug-Score	0.20

(7)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

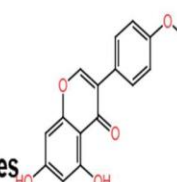
cLogP	-0.75
Solubility	-0.54
Molweight	278.37
TPSA	93.07
Druglikeness	-11.08
H bond acceptor	6
H bond donor	2
Nb stereocenters	0
Nb rotatable bonds	0
Drug-Score	0.29

(8)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	1.90
Solubility	-3.04
Molweight	284.27
TPSA	75.99
Druglikeness	1.02
H bond acceptor	5
H bond donor	2
Nb stereocenters	0
Nb rotatable bonds	2
Drug-Score	0.82

(9)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	2.72
Solubility	-2.54
Molweight	146.23
TPSA	0.00
Druglikeness	-1.36
H bond acceptor	0
H bond donor	0
Nb stereocenters	2
Nb rotatable bonds	0
Drug-Score	0.34

(10)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

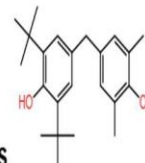
cLogP	5.33
Solubility	-3.60
Molweight	240.39
TPSA	26.30
Druglikeness	-30.91
H bond acceptor	2
H bond donor	0
Nb stereocenters	0
Nb rotatable bonds	12
Drug-Score	0.28

(11)

OSIRIS Property Explorer

Predicted toxicity risks

■ mutagenic
■ tumorigenic
■ irritant
■ reproductive effective



Predicted properties

cLogP	6.64
Solubility	-5.74
Molweight	340.51
TPSA	40.46
Druglikeness	-21.49
H bond acceptor	2
H bond donor	2
Nb stereocenters	0
Nb rotatable bonds	4
Drug-Score	0.27

(12)

Figure 5.10: this figure represents the non-toxic compound results obtained from the OSIRIS online tool.

- (1) Curan-17-oic acid,19,20-didehydro,-14-hydroxy-methyl ester
- (2) Cyclododecanecarboxylic acid, methyl ester
- (3) Dodecanoic acid,11-oxo-methyl ester
- (4) Isopropyl stearate
- (5) Octadec-9-enoic acid
- (6) Oleic acid
- (7) Z-13-octadecen-1yl acetate
- (8) 1,4,7,10-tetraox-13,15-diazacycloheptadecane-14thione
- (9) 4' methoxy-5,7-dihydroxy flavone
- (10) 7-ethylidenebicyclo (4,2,1) nona2,4-diene
- (11) Methyl Z-11-tetradecenoate
- (12) Phenol,2,6-bis(1,1-dimethyl ethyl)-4-[(4-hydroxy-3,5-dimethyl phenyl)]

4.17.2 ADMET profiling

The ADMET profiling is used to find the drug likeness of the compounds. The better compounds can be identified by applying Lipinski rule. The rule is applied when molecular weight of the compounds should be less than 500 Daltons. Compounds should express high lipophilicity and contain no more than 5 hydrogen bond donors and have less than 10 hydrogen bond acceptors. Molar refractivity should be 40-130. Out of 22 compounds from both samples only 2 compounds were selected for drug likeness and can be further used for the docking.

cLogP		1.90
Solubility		-3.04
Molweight		284.27
TPSA		75.99
Druglikeness		1.02
H bond acceptor		5
H bond donor		2
Nb stereocenters		0
Nb rotatable bonds		2
Drug-Score		0.82

(1)- 4' methoxy-5,7-dihydroxy flavone

cLogP		1.15
Solubility		-2.84
Molweight		340.42
TPSA		61.80
Druglikeness		1.30
H bond acceptor		5
H bond donor		2
Nb stereocenters		6
Nb rotatable bonds		2
Drug-Score		0.83

(2)- Curan-17-oic acid,19,20-didehydro,-14-hydroxy-methyl ester

Figure 5.11: This figure represents the drug-likeness parameters for two compounds that have good drug-score properties.

4.18 Antibacterial assay

The antibacterial assay was carried out with both extract and crude as well as the elutes obtained from the column chromatography. The eluted samples were lyophilized and mixed with DMSO and added to each well. The 9-plant crude shows a mild zone in both *Bacillus cereus* and *Staphylococcus aureus*. No zone was found in the *E. coli* culture plates.

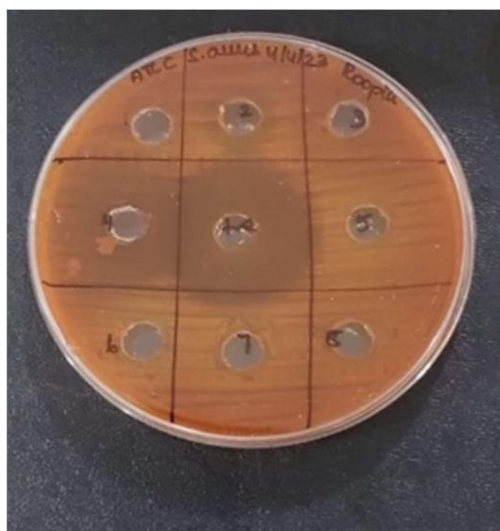


E. coli plate- 1
Mueller- Hinton agar

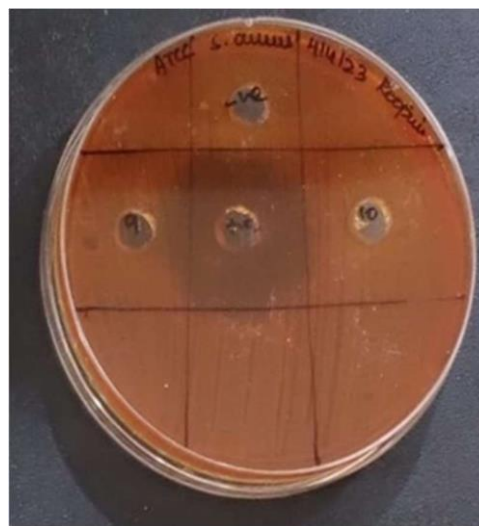


E. coli plate -2
Mueller- Hinton agar

Figure 5.12: This picture represents the antibacterial activity of the samples by using the *E. coli* strain.

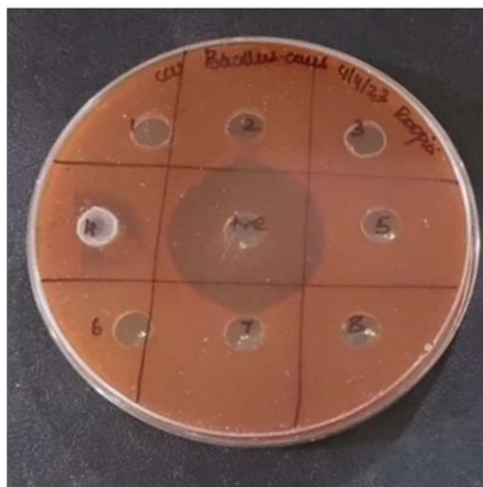


Staphylococcus aureus plate- 1
Mueller- Hinton agar



Staphylococcus aureus plate- 2
Mueller- Hinton agar

Figure 5.13: This picture represents the antibacterial activity of the samples by using the *Staphylococcus aureus* strain.



Bacillus cereus plate- 1
Mueller- Hinton agar



Bacillus cereus plate- 1
Mueller- Hinton agar

Figure 5.14: This picture represents the antibacterial activity of the samples by using the *Bacillus cereus* strain.

4.19 Antimycobacterial assay

The antimycobacterial assay was carried out with both extract and crude as well as the elutes obtained from the column chromatography. The eluted samples were lyophilized and mixed with DMSO and added to each well. Mostly all the plant extract and crude show mild inhibition in each well. This shows that both samples may tend to inhibit the *Mycobacterium smegmatis*.



Mycobacterium smegmatis plate- 1
Middlebrook 7H11 agar



Mycobacterium smegmatis plate- 2
Middlebrook 7H11 agar

Figure 5.15: This picture represents the antimycobacterial activity of the samples by using the *Mycobacterium smegmatis* strain.

4.20 MABA Assay

The MABA assay was carried out with two different samples S1 and S2. For both samples crude and extracts were used in the different concentrations. After adding Alamar blue, the colour changes to pink colour, indicating the samples does not have any activity against the *Mycobacterium smegmatis*

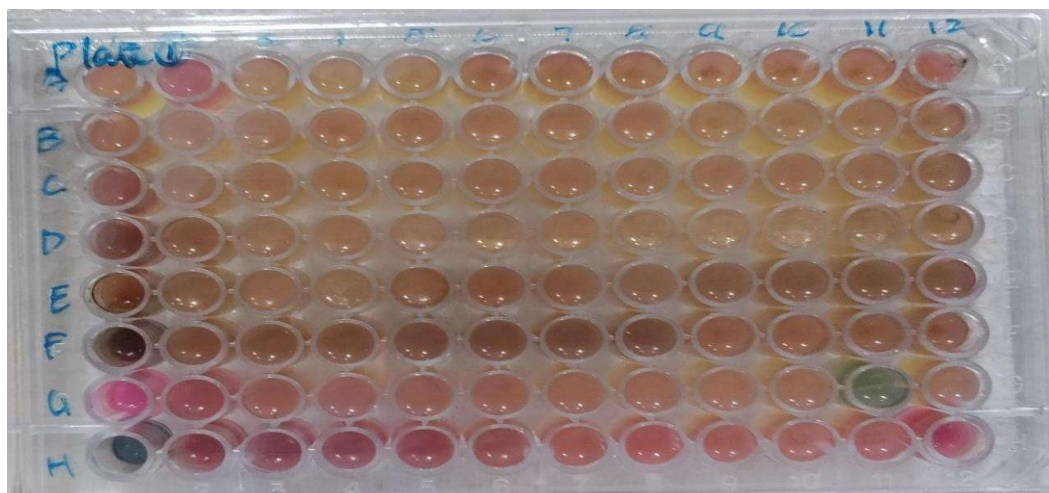


Figure 5.16: This figure represents the MABA Assay performed in 96 well plate.

4.21 Cytotoxicity assay

Cytotoxicity of obtained crude samples 1 and 2 was performed using an MTT assay. Absorbance was measured at 570 nm using an ELISA reader. The results and graph were interpreted and mentioned below

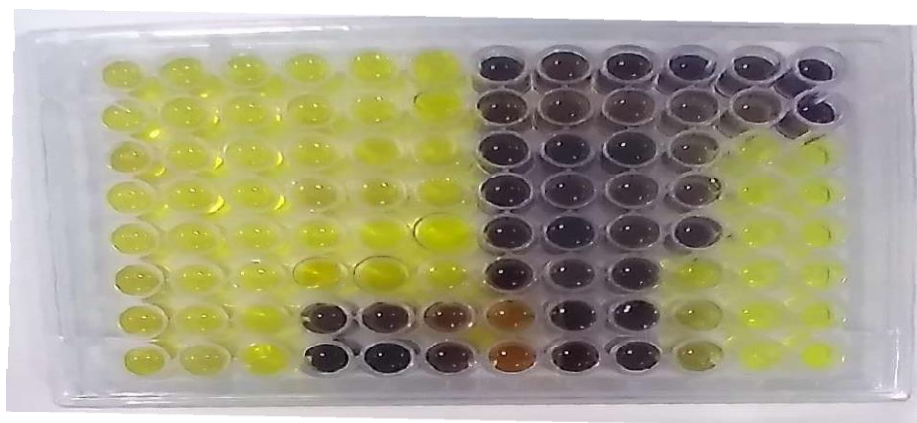


Figure 5.17: This figure represents MTT assay carried out for both samples

Sample number	OD at 540nm	% viability
<i>Coleus ambionicus</i> crude	0.77	61.57
	0.53	40.44
	0.30	19.67
	0.09	1.57
	0.07	0.20
	0.08	1.11
9 plant crude	0.92	74.59
	0.84	67.31
	0.17	8.33
	0.07	0.09
	0.07	-0.15
	0.09	1.81
<i>Coleus ambionicus</i> extract	1.13	92.80
	1.26	103.76
	1.33	110.02
	1.02	82.95
9 plant extract	1.23	101.46
	1.07	87.38
	0.95	77.24
	1.31	108.13

Table 5.8: This table represents the percentage viability of each sample in different concentrations.

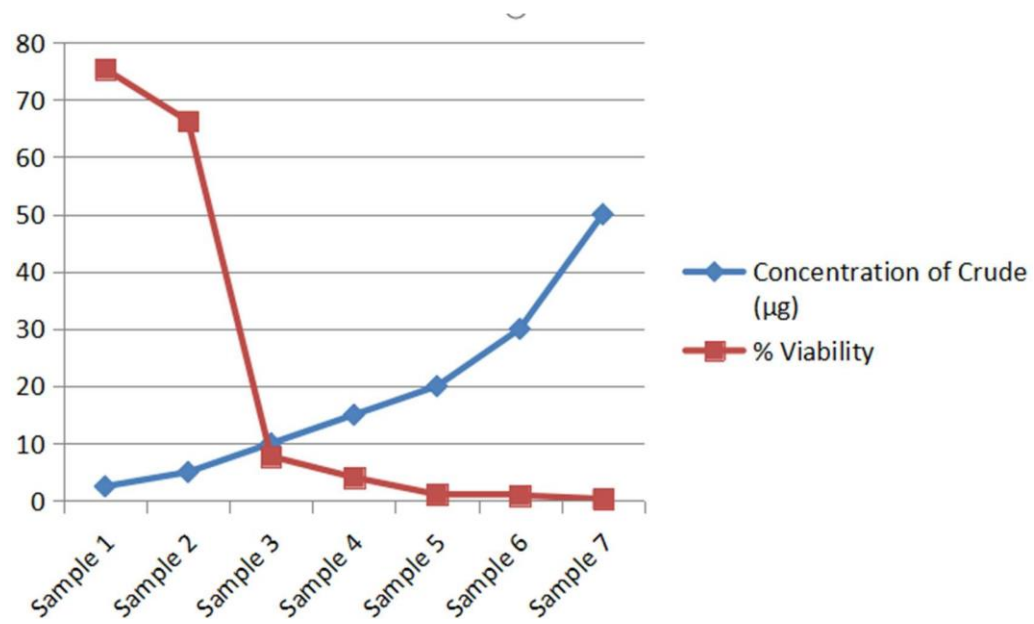


Figure 5.18: This graph represents that when the concentration increases then the cell viability decreases.

Discussion

The literature survey on the antiviral properties of various medicinal plants has exerted a huge identification of the plant compounds and their mechanistic action against viruses. The compounds and antiviral assays of all the plants were noted and separated the plants which have more phenolic compounds in their extract. This is because SARS-CoV-2 and *Mycobacterium tuberculosis* cause respiratory tract infections. To control those infections the phenolic compounds effectively enter the passage of the upper respiratory system and may potentially inhibit viral and bacterial infections. So, the nine medicinal plants that have been selected for the project are found to have aromatic compounds present in it. The plant *Coleus amboinicus* have been individually analysed along with the combination of 9 plants. These both *Coleus amboinicus* and 9 plant combinational plant extracts have the similar Phyto molecules such as Saponins, alkaloids, Quinones, Flavonoids, Tannins, Alkaloids, and steroids. The free radical scavenging property of the plant *Coleus amboinicus* extract has maximum of 93% inhibition whereas the 9-plant combinational extract has a 93.51% inhibition maximum and the extracts of both samples show a maximum haemolytic property. Around 60% of the blood get lysed in the 9 plant combinational extract in higher volume. Computationally these plant extracts were checked for toxicity and drug-likeness. The compounds namely Curan-17-oic acid, 19,20-didehydro-, 14-hydroxy-methyl ester and 4' methoxy- 5,7-dihydroxy flavone were found to have compatible drug scores. In the MABA assay the extracts were not found to have the compatible inhibiting property in MABA assay.

And in invitro conditions when the concentration of the drug increases the viability of the cells gets decreased. Hence these plants may have the intensity to produce as drug molecules against these pathogens.

Chapter 6

SUMMARY AND CONCLUSION

Medicinal plants have been used for their therapeutic values. More than 8000 medicinal plant species are there in India. Various diseases can be diagnosed and prevented by using medicinal plants which have high therapeutic value. The major problem is that SARS-CoV-2 lacks proper medication and treatment, so medicinal plants were used to treat these viruses and mycobacterial infections. Medicinal plants generally do not have any side effects since they are naturally available sources. So, the compounds extracted from the plants may have the potential to inhibit these two pathogens and the GCMS report obtained from these two samples has 22 compounds. Out of these 16 compounds are found to be non-toxic and from 16 compounds 2 compounds namely Curan-17-oic acid, 19,20-didehydro-, 14-hydroxy-methyl ester, and 4'-methoxy-5,7-dihydroxy flavone are found to have good drug score. Further the activities of these compounds could be analyzed to formulate these two samples into lead drug molecules against SARS CoV-2 and *Mycobacterium tuberculosis*. Since these plants have aromatic compounds present in it. Hence in further studies it could be formulated into inhaler or capsule that could migrate the compounds effectively via the inhalation process.

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