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8. Sprouts as Novel Anti-Fungal Agents against Isolates from Air Conditioner Remote and Docking Studies against Aspergillus Niger

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Abstract:

Air conditioners and Air conditioner remotes acts as sources of infections caused by microbes. Sprouts are cheap, easily prepared, and safe and composed of multiple active constituents to combat life threatening infectious diseases caused by microorganisms. The main aim of this study was to investigate the level of fungal contamination and determination of antifungal activity of crude extracts of sprouts followed by docking studies. Seeds such as Lentils, Mung bean, Chick pea, Field pea and Green peas were used for germination of Sprouts. The aqueous crude extracts of sprouts were prepared. The fungal isolates were identified as A. Niger, A. flavus and Rhizopus. The fungal isolates from AC remotes were screened by using aqueous crude extracts of sprouts by agar well diffusion technique at different concentrations. Mung bean sprout aqueous extract was found to most effective. The MIC of the Mung Bean aqueous sprout extract to inhibit the sensitive strain was found to be 0.125µg/ml. The RF values of TLC of Mung Bean were 0.52, 0.21 and 0.39 under iodine stain and UV. The docking studies reported that Gallic acid of Mung Bean and Endo- Polygalacturonase II of A Niger has more binding interaction to inhibit the fungal cell wall with binding energy of – 4.92

Keywords:

Aqueous extracts, MIC, Docking studies, Gallic acid, Endo- Polygalacturonase II, Sprouts.

8.1 Introduction:

Air conditioners serves as sources of microbial infections. AC makes indoor environment sterile but due to personal unhygienic conditions and dust may lead to bronchitis, rhinitis, keratitis, conjunctivitis, pharyngitis and pneumonia. Indoor air quality is directly propositional to the microbial growth due to changes temperature, relative humidity and

high temperature. The reported organisms in air-conditioned filter are Legionella pneumophilla, Streptococcus pneumonia and Bacillus spp, Penicillium pneumocystis, Carinii and species of Aspergillus, Rhizopus, Fusarium and Alternaria. The aim of this study was to reveal the level of bacterial and fungal contamination by using of Air conditioner remotes AC and its remote spread the diseases in such environments like long-term care facilities, sports facilities and hospitals.

Different types of microorganisms are identified includes rhinovirus, rotavirus, Serratia marcescens and methicillin- resistant staphylococcus aureus (MRSA) these organisms causes common cold, catheter associated bacteremia, necrotizing facilities, gastrointestinal diseases. Staphylococcus causes infections to diabetic patients, immunocompromised patients, new borns, surgical and burns. The sources of infection are hands, towels, touched objects

Sprout is a major source of protein and constituents an important health food in wide variety of Indian traditional foods. Sprouts are economically feasible and preparation is possible with minimum efforts. Sprouts can be prepared using vegetables and grains, cereals, pulses, etc. Sprouts play an important role in promoting human health. Sprouts make an important nutritive supplement for majority of people suffering from diabetes, thyroid, obesity, arthritis and infectious disease caused by microorganisms. Microbial infections are most common in crowded communities, immunocompromised people leading to the major outbreaks in several countries. Most of the microbes are becoming resistant to multiple antibiotics leading to the development of multidrug resistant bacteria such as Escherichia. Coli, Salmonella spp, Vibrio cholera, Shigella spp, Staphylococcus spp, Bacillus spp, Pseudomonas spp, Aspergillus spp, Candida spp and Actinomycetes.

The sources of these microbial infections are due to improper hygienic conditions, aerosol, water and food. The route of infection might include ingestion, inhalation and contact. As microbes are becoming resistant to multidrug and choice of antibiotic treatment is troublesome hence, there is a need to troubleshoot this problem by selecting an alternative treatment to overcome chemoprophylaxis. Sprouts are cheap, easily prepared, safe and composed of multiple active constituents to combat life threatening infectious diseases caused by microorganisms. The prevention and reduction of cross contamination and spread of infections could be done by proper washing of hands using Hand wash.

8.2 Materials and Methods:

a. Collection of Seeds:

Seeds such as Lentils, Mung bean, Chick pea, Field pea and Green peas were bought and allowed for germination of sprouts.

b. Germination of Sprouts:

The seeds were soaked in drinking tap water and kept in plastic box covered with aluminum or silver foil followed by germination of sprouts for one week at room temperature under aseptic condition.

c. Preparation of Extracts from Sprouts:

- The sprouts were separated from seeds aseptically using sterile disposable gloves and spread in sterile filter paper to absorb excess of moisture.
- He sprouts were dried under shade, powdered and soaked in sterile distilled water for overnight in sterile flask.
- The respective flask was subjected to filtration using sterile whatmann filter paper.
- The filtrates of respective sprouts were centrifuged to obtain supernatant consisting of Bioactive components.
- The residue was discarded and supernatant from respective flask were transferred aseptically into sterile flask and filtrates were stored at 4°c for antimicrobial activity.
- The sprouts were separated from seeds aseptically using sterile disposable gloves and spread in sterile filter paper to absorb excess of moisture.
- The sprouts were dried under shade, powdered and soaked in sterile distilled
- Water for overnight in sterile flask.
- The respective flask was subjected to filtration using sterile whatmann filter paper.
- The filtrates of respective sprouts were centrifuged to obtain supernatant consisting of Bioactive component

Figure 8.1: Preparation of sprout extracts from seeds- Lentils, Mung Bean, Green Pea, Chick Pea and Field Pea

Collection of Air conditioner remote samples: The non-clinical samples were collected from Air condition remotes. The fungal isolates were isolated and identified based on morphological and biochemical characters.

Figure 8.2: Preparation of Inoculum

The fungal strains such as Aspergillus Niger, Aspergillus flavus and Rhizopus species were inoculated into potato dextrose broth and incubated for 30 minutes.

d. Screening of Antimicrobial Activity of Sprouts of Lentil, Mung Bean, Green Pea, Chick Pea and Field Pea:

Anti-fungal activity of sprouts of lentil, mung bean, green pea, chick pea and field pea were determined investigated by agar well diffusion method. Sterile cotton swabs were used for preparation of lawn. Muller Hinton agar plates were prepared and lawn was done on punched wells the wells were filled with different concentration (1000µl, 500µl, 250µl and 125µl) of liquid extracts of respective sprouts for all the fungal isolates. The plates were incubated and plates were absorbed for inhibitory zones. Minimum inhibitory concentration was determined followed by thin layer chromatography. Auto Docking was performed to find out binding energy of compound of sprout with fungal cell wall protein.

Figure 8.3: Preparation of Lawn Using Fungal Isolates

Figure 8.4: Broth dilution

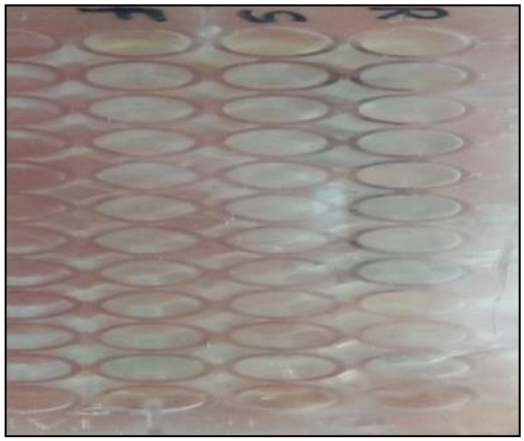


Figure 8.5: Micro dilution technique MIC

e. Result and Discussion

- **Fungal Identification**

Table 8.1: Fungal Identification

Sr. No	Fungi Name	Cultural Characters	Morphological Characters
1	*Aspergillus niger*	Woolly, Dark brown to black	Conidiophores wit variable length, phalides are biseriate
2	*Aspergillus flavus*	Velvety, Yellow to green or brown	Conidiophores with variable length, rough, pitted are single and double cover entire vesicle with point out various direction
3	*Rhizopus*	Dense cottony growth, becoming grey or yellowish brown	Stolon and pigment rhizoid, sporangiospores are globose to ovoid, one celled hyaline to brown and striate

f. *Aspergillus Niger*:

i. Lentil Aqueous Sprout Extract:

Table 8.2: Lentil aqueous sprout extract

Sr. No	1000µl	500µl	250µl	125µl
R2	++	+	-	-

The highest activity was found to be good at 1000µl as compared to 500 µl concentration of Lentil sprout extract.

ii. Mung Bean Aqueous Sprout Extract:

Table 8.3: Mung Bean aqueous sprout extract

Sr. No	1000µl	500µl	250µl	125µl
R2	+++++++	++++	+++	++

The highest rate of inhibition was found to be excellent and most promising at 1000µl followed by zone of inhibition at 500 µl & 250 µl and minimum rate of inhibition at 125 µl of Mung Bean aqueous extract.

iii. Green Pea Aqueous Sprout Extract:

Table 8.4: Green Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R2	++++++	++++	+++	++

The maximum rate of inhibition was found to be excellent at 1000 µl of Green pea aqueous sprout extract and minimum inhibition at 125 µl.

iv. Chick Pea Aqueous Sprout Extract:

Table 8.5: Chick Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R2	+++	++	+	-

The zone of inhibition was found to be very good at 1000µl of Chick Pea aqueous sprout and showed no zone of inhibition at 125 µl concentration of extract.

v. Field Pea Aqueous Sprout Extract:

Table 8.6: Field Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R2	+++	++	++	+

The zone of inhibition was found to be good at 1000 µl and least rate of inhibition at 125 µl of Field Pea aqueous sprout extract.

g. *Aspergillus flavus:*

vi. Lentil Aqueous Sprout Extract:

Table 8.7: Lentil aqueous sprout extract

Sr. No	1000µl	500µl	250µl	125µl
R1	+++	++	-	-

Lentil aqueous sprout extract showed maximum zone of inhibition at 1000 µl concentration and was found to show no zone of inhibition at 125 µl concentration of lentil sprout extract for *Aspergillus flavus*.

vii. Mung Bean Aqueous Sprout Extract:

Table 8.8: Mung Bean Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R1	++++	++	+	+

The zone of inhibition for Mung Bean aqueous extract was found to be excellent at 1000µl concentration when compared to 125 µl which showed least rate of inhibition.

viii. Green Pea Aqueous Sprout Extract:

Table 8.9: Green Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R1	+++	+++	+	+

Green aqueous sprout extract exhibited very good rate of inhibition at 1000 µl of concentration and low rate of inhibition at 125 µl concentration.

ix. Chick Pea Aqueous Sprout Extract:

Table 8.10: Chick Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R1	+	-	-	-

The zone of inhibition was found to be very low at 1000 µl followed by no zone of inhibition at 500 µl to 125 µl concentrations of the aqueous extract.

x. Field Pea Aqueous Sprout Extract:

Table 8.11: Field Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R1	+	-	-	-

The zone of inhibition was found to be very low at 1000 µl.

Followed by no zone of inhibition at 500 µl to 125 µl concentrations of the aqueous extract.

h. *Rhizopus Spp:*

xi. Lentil Aqueous Sprout Extract:

Table 8.12: Lentil Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R2	++++	+++	+++	++

Lentil sprout aqueous extract showed excellent rate of inhibition for Rhizopus spp at 1000µl and low rate of inhibition at 125 µl of concentration.

xii. Mung Aqueous Bean Sprout Extract:

Table 8.13: Green Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R2	+++++	++++	++	+

The zone of inhibition was very high and most potent at 1000 µl concentration and least at 125 µl concentration of Mung Bean aqueous sprout extract**.**

xiii. Green Pea Aqueous Sprout Extract:

Table 8.14: Green Pea Aqueous Sprout Extract

Sr. No	1000µl	500µl	250µl	125µl
R2	++++	+++	++	+

The zone of inhibition was found to be maximum at 1000µl concentration of aqueous Green pea extract and minimum at 125 µl concentration.

xiv. Chick Pea Aqueous Sprout Extract:

Table 8.15: Chick pea aqueous sprout extract

Sr. No	1000µl	500µl	250µl	125µl
R2	+++	+	+	-

The zone of inhibition for Rhizopus spp was found to be very good at 1000 µl of Aqueous Chick pea extract and no zone of inhibition was observed at 125 µl concentration. Field pea aqueous sprout extract

Table 8.16: aqueous extracts of Field Pea

Sr. No	1000µl	500µl	250µl	125µl
R2	++++	+++	+++	+++

The rate of inhibition was found to be maximum at 1000µl and showed moderate levels of inhibition at low concentrations of aqueous extracts of Field Pea.

Figure 8.6: ***Aspergillus Niger***

Figure 8.7: A. niger on SDA

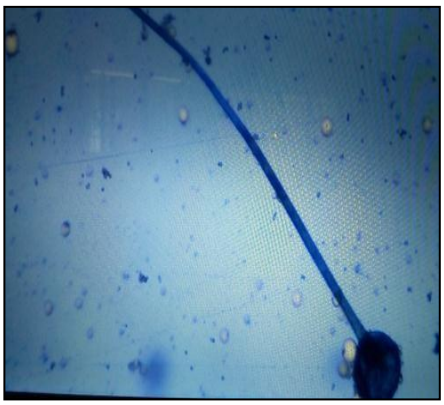


Figure 8.8: ***A.flavus***

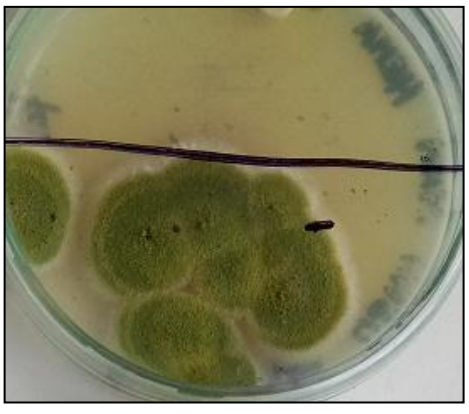


Figure 8.9: A. flavus on SDA

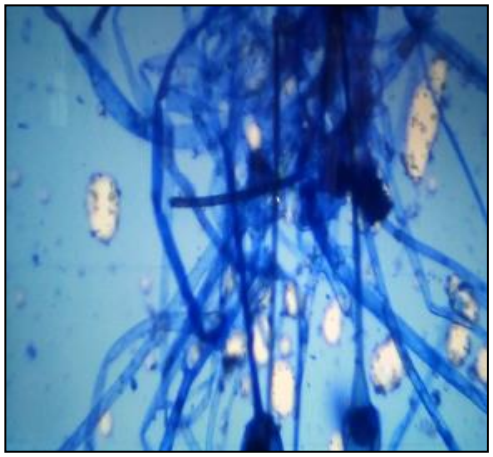


Figure 8.10: Rhizopus spp

Figure 8.11: Rhizopus spp on SDA

xv. Anti-fungal Activity of Lentil, Mung Bean, Green Pea, Chick Pea and Field Pea Aqueous sprout extracts- Aspergi
xvi. llus Niger

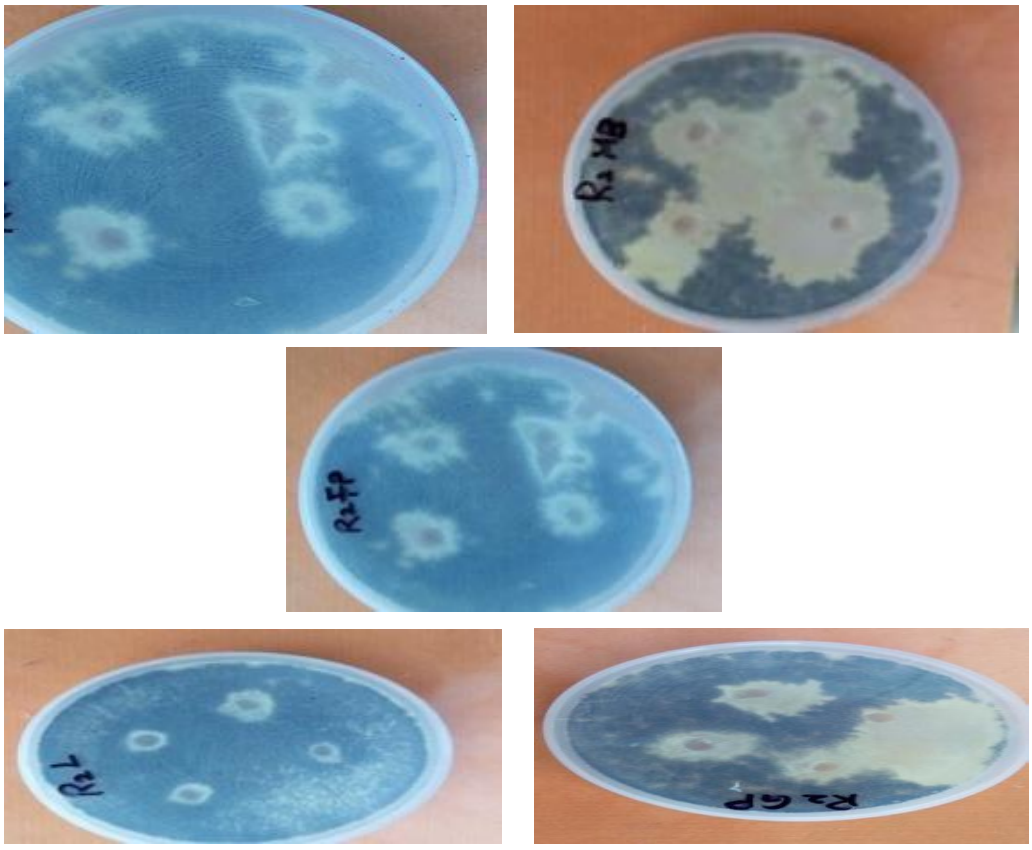


Figure 8.12: Anti-fungal Activity of Lentil, Mung Bean, Green Pea, Chick Pea and Field Pea Aqueous sprout extracts- Aspergillus Niger

xvii. Anti-fungal Activity of Lentil, Mung Bean, Green Pea, Chick Pea and Field Pea Aqueous sprout extracts — Aspergillus flavus

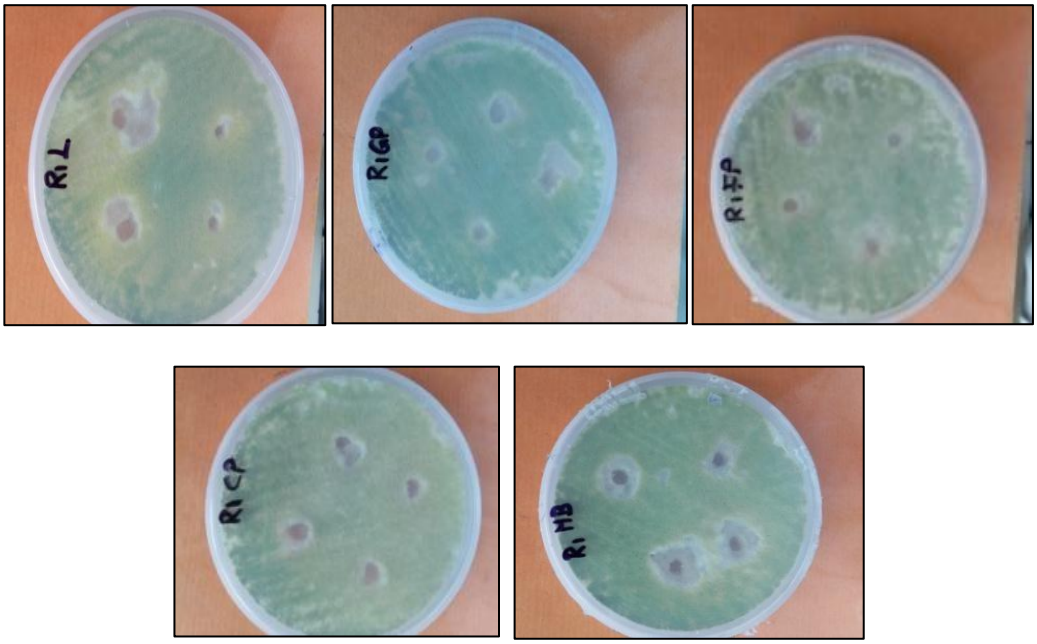


Figure 8.13: Aqueous sprout extracts — Aspergillus flavus

xviii. Anti-fungal Activity of Lentil, Mung Bean, Green Pea, Chick Pea and Field Pea aqueous sprout extracts

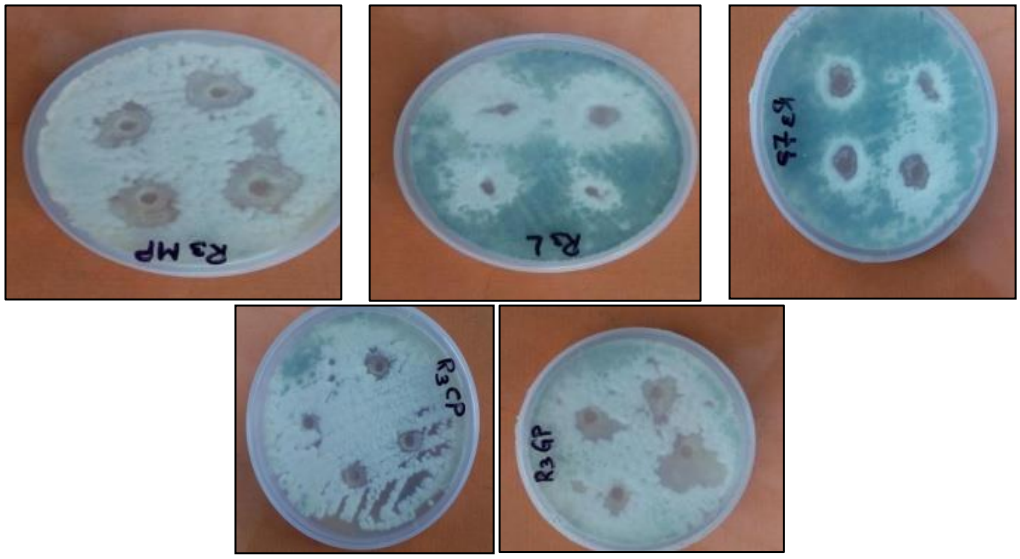


Figure 8.14: Anti-fungal Activity of Lentil, Mung Bean, Green Pea, Chick Pea and Field Pea aqueous sprout extracts

xix. Thin layer Chromatography- Mung bean aqueous sprout extract

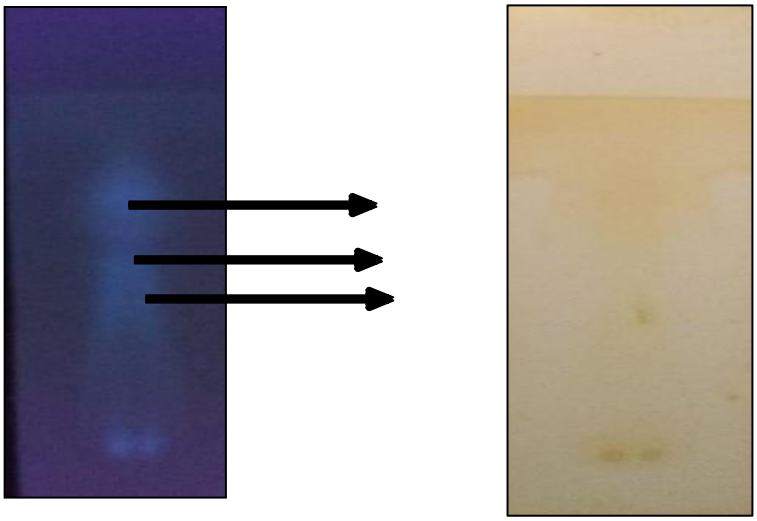
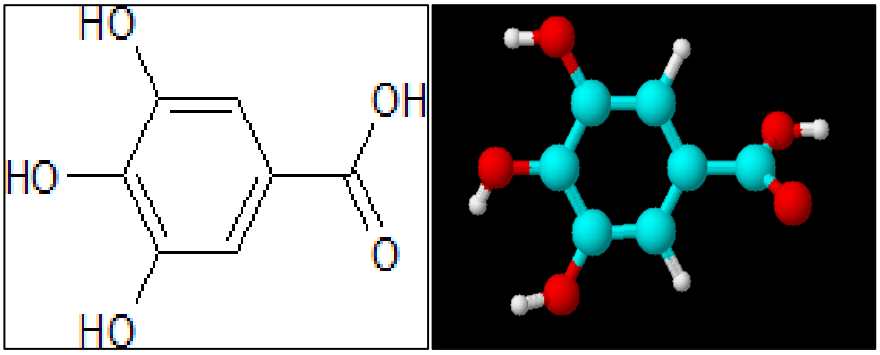


Figure 8.15: Thin layer Chromatography

i. Bioinformatics:

xx. Gallic acid



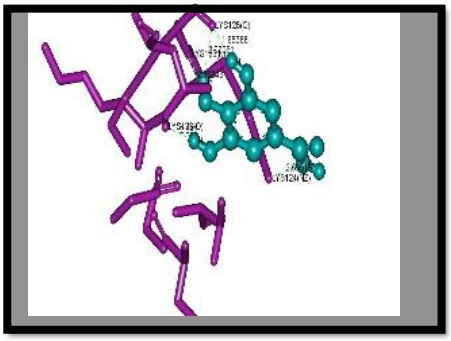


Figure 8.16: Gallic acid

xxi. Visualizing hydrogen interactions between Gallic acid and endo-Polygalacturonase -Discovery studio visualizer

Table 8.17: Visualizing hydrogen interactions between Gallic acid and endo-Polygalacturonase

Endo- Polygalacturonase II		Gallic acid	Distance (Å)	Docking Energy (Kcal/Mol)
Residue	Atom			
LYS123	O	H	2.08	-4.92

The current study was focused on anti-fungal activity using aqueous extract of sprouts. The highest activity was found to be good at 1000µl as compared to 500 µl concentration of Lentil sprout extract. The highest rate of inhibition was found to be excellent and most promising at 1000µl followed by zone of inhibition at 500 µl &250 µl and minimum rate of inhibition at 125 µl of Mung Bean aqueous extract.

The maximum rate of inhibition was found to be excellent at 1000 µl of Green pea aqueous sprout extract and minimum inhibition at 125 µl. The zone of inhibition was found to be very good at 1000µl of Chick Pea aqueous sprout and showed no zone of inhibition at 125 µl concentration of extract.

The zone of inhibition was found to be good at 1000 µl and least rate of inhibition at 125 µl of Field Pea aqueous sprout extract against A. Niger. Lentil aqueous sprout extract showed maximum zone of inhibition at 1000 µl concentration and was found to show no zone of inhibition at 125 µl concentration.

The zone of inhibition for Mung Bean aqueous extract was found to be excellent at 1000µl concentration when compared to 125 µl which showed least rate of inhibition. Green aqueous sprout extract exhibited very good rate of inhibition at 1000 µl of concentration and low rate of inhibition at 125 µl concentration.

The zone of inhibition was found to be very low at 1000 µl followed by no zone of inhibition at 500 µl to 125 µl concentrations of the aqueous Chick Pea extract. The zone of inhibition was found to be very low at 1000 µl followed by no zone of inhibition at 500 µl to 125 µl concentrations of the aqueous Field Pea sprout extract against A. flavus. Lentil sprout aqueous extract showed excellent rate of inhibition for Rhizopus spp at 1000µl and low rate of inhibition at 125 µl of concentration.

The zone of inhibition was very high and most potent at 1000 µl concentration and least at 125 µl concentration of Mung Bean aqueous sprout extract. The zone of inhibition was found to be maximum at 1000µl concentration of aqueous Green pea extract and minimum at 125 µl concentration. The zone of inhibition for Rhizopus spp was found to be very good at 1000 µl of aqueous Chick pea extract and no zone of inhibition was observed at 125 µl concentration. The rate of inhibition was found to be maximum at 1000µl and showed moderate levels of inhibition at low concentrations of aqueous extracts of Field Pea against Rhizopus spp.

8.3 Conclusion:

The present study revealed that the minimum concentration of the Mung Bean aqueous sprout extract to inhibit the sensitive strain was found to be 0.125µg/ml of the extract. This study reported that the RF values of TLC of Mung Bean are 0.52, 0.21 and 0.39 under iodine stain and UV. The docking studies revealed that Gallic acid of Mung Bean and Endo-Polygalacturonase II of A Niger has more binding interaction to inhibit the fungal cell wall protein.

8.4 Acknowledgement:

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