X.A - Antimicrobial activity

From ancient age, plants or its parts are used for medicinal purposes. It has seen that the African gorillas used various plants parts for curing themselves (Tripathi and Mondal, 2012). In Country like India, 80% people lives in village areas and the treatment of that area are dependent on folklore medicines. This type of medicine derived from Unani system of medicines (Somro, et al; 1997). Plants are the most common source of treating agents. The traditional health remedies are very much popular throughout the world. In south west Bengal, Tribal people are often used plants and their different parts for treatment against common infectious diseases and the plant materials used for traditional medicine are more available and relatively cheaper than the modern medicine. Some hemi parasites effective as Antimicrobial agents is also reported (Hussainn, et al; 2011).

Materials and Methods

Plants and its different parts were collected from the different areas. The information regarding their different medicinal uses, procedures of uses are documented by continuous observation and simultaneous interaction with local villagers.

Collection and preparation of plant material for extraction

Plant parts dissolved in 70% alcohol and rinsed by sterilized distilled water. After that the materials are air dried, homogenized with mixer grinder and stored in cotton bags.

Preparation of Methanol Extracts

10 g of powdered material of sample was soaked in 30 ml of 70% methanol and kept at 37°C for 24 h on a rotary shaker. It was then filtered with the help of a Whatman No. 1 filter paper. The filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was

then collected and dried to evaporate. Then it was preserve for further uses (Ushimaru, et al; 2007).

Fig – 78: Rotary shaker

Preparation of Aqueous Extracts

Same procedure is followed instead sterilized distilled water is used in case of methanol (Das, et al; 2011).

Preparation of Acetone extracts

Same procedure is followed instead sterilized distilled acetone is used in case of methanol.

The extracts were kept in sterile air tight bottles at 4°C until further use. Before use, 30 mg of dry extract was re-suspended in 1 ml of acetone so that the final concentration of the extract was 30 mg/ml.

Fig – 79: Extract filter with Whatman no.1 filter paper

Fig – 80: Extract with in sterile air tight bottles

Bacterial strains

Pure cultures of four bacterial strains ***Bacillus subtilis*** (Cohn, 1872)***, Escherichia coli*** (Castellani and Chalmers, 1979)***, Klebsiella pneumonia*** (Trevisan, 1887) ***and Aeromonas hydrophila*** (Stainer, 1943) were used for the study.

Agar well diffusion

Antimicrobial activity was determined by the agar-well diffusion method.

X.A.1.i - Results (*Loranthus parasiticus*)

The result of screening plant extracts for antimicrobial activity was summarized in the Table No.10, 11 & 12. It was found that the plant extract had antimicrobial activity of different degrees. **Aqueous** extract of *Loranthus parasiticus* shows, after 24 hrs. incubation that *Loranthus parasiticus* produced inhibition zone against Bacillus subtilis (7.5mm), Klebsiella pneumoniae (10.5mm) and Escherichia coli (9mm). But in case of *Aeromonas hydrophila* inhibition zone was (10mm). But in case of **Methanolic** extract of *Loranthus*

parasiticus show inhibition zone of *Bacillussubtilis* (7.5mm), *Klebsiella pneumoniae*(12mm), *Escherichia coli* (12mm), *Aeromonus hydrophila* (13.5), so in case of methanoic extract the inhibition zone is highest of *A. hydrophila*. In case of **Acetone** extract the inhibition zone is highest of *A.* hydrophila (14mm) against other three bacterial strain, *Bacillus subtilis* (8mm), *Escherichia coli* (10mm), *K. pneumoniae* (12mm).

After 48 hrs, Incubation it was found that the previously formed inhibition zones were increased in all bacterial strain in case of **aqueous** extract. *Escherichia coli* (10 mm), *Klebsiella pneumoniae* (12 mm), *Bacillus subtilis* (9mm) &*Aeromonas hydrophila* (13.5 mm). But in case of **Acetone** extract the inhibition zone are unchanged of *E. coli* and another strain are increase *K. pneumoniae* (15mm), *B. subtilis* (10mm), *A. hydrophilla* (15mm) and *E. coli* (14mm), *K. pneumoniae*. (14mm), *B. subtilis* (10mm), *A. hydrophilla* (15mm) in case of **Methanol** extract of *Loranthus parasiticus* plant.

In this experiment, if we compatible with four bacterial strain about their inhibition capabilitywe found that, in case of Aquous extract after 24 hour- *K. pneumoniae>A. hydrophila>E. coli>B. subtilis* and after 48 hours of incubation, *A. hydrophila>K. pneumoniae>E. coli>B. subtilis*. In case of Methanol extract- after 24 hours incubation, *A. hydrophila>E. coli = K.pneumoniae>B. subtilis* and after 48 hours *A. hydrophila>E. coli = K. pneumoniae>B. subtilis*. In case of acetone extract, after 24 hours incubation *A. hydrophila >K. pneumoniae>E. coli>B. subtilis*, after 48 hours incubation the order is *A. hydrophila = K. pneumoniae>E. coli = B. subtilis*.

Table - 22: Antimicrobial effect of the **Methanol extracts** of the *Loranthusparasiticus*.

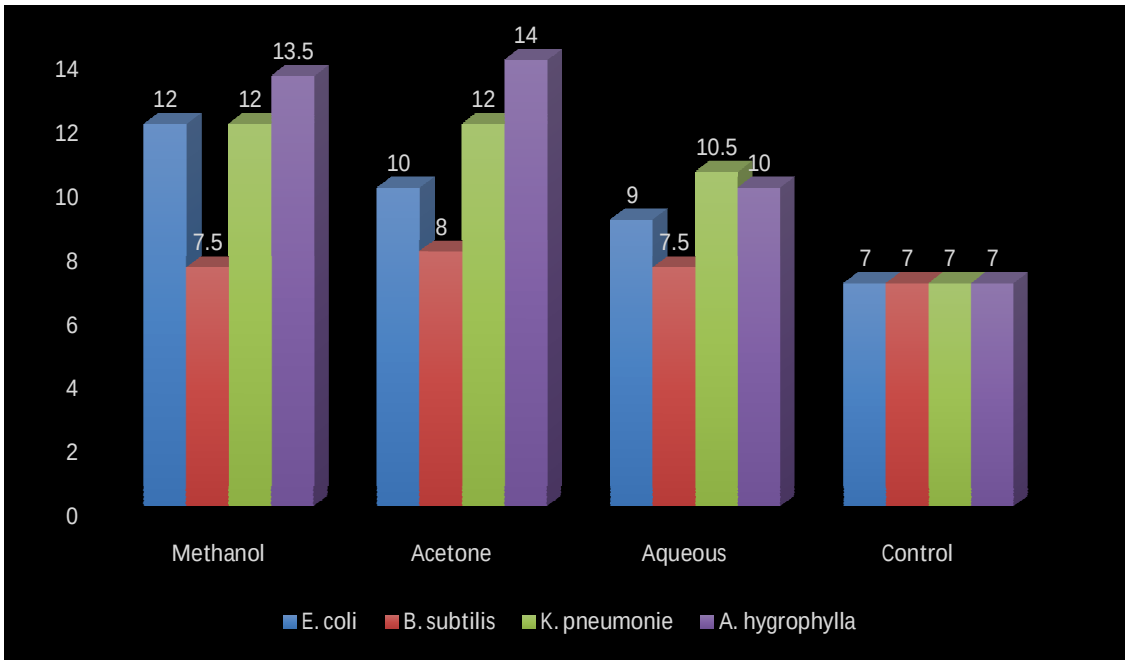
Plant	Diameter of the inhibition zone(mm)							
Loranthus Parasiticus	***Escherichia coli***		***Klebsiella pneumoniae***		***Bacillus subtilis***		***A hydrophila***	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	12	14	12	14	7.5	10	13.5	15

Table - 23: Antimicrobial effect of the **Acetone extracts** of the *Loranthus parasiticus*

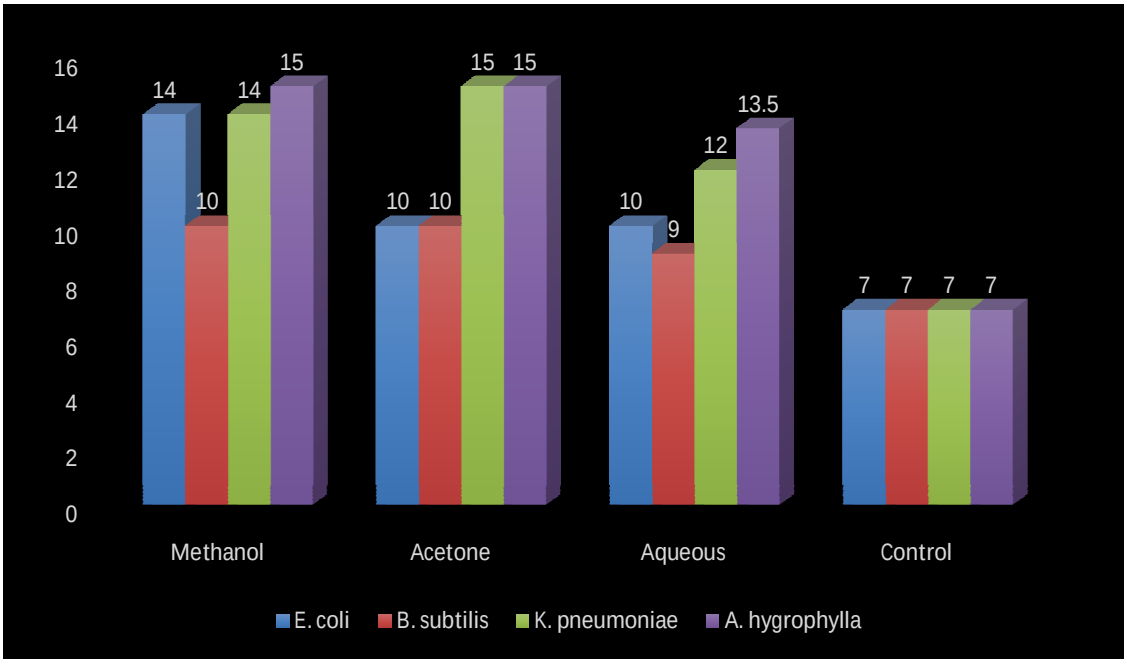
Plant	Diameter of the inhibition zone(mm)							
Loranthus Parasiticus	***Escherichia coli***		***Klebsiella pneumoniae***		***Bacillus subtilis***		***A hydrophila***	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	10	10	12	15	8	10	14	15

Table - 24: Antimicrobial effect of the **Aqueous extracts** of the *Loranthus parasiticus*

Plant	Diameter of the inhibition zone(mm)							
Loranthus Parasiticus	***Escherichia coli***		***Klebsiella pneumoniae***		***Bacillus subtilis***		***A hydrophila***	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	9	10	10.5	12	7.5	9	10	13.5



Graph - 7: Antimicrobial effect on the *L. parasiticus* plants (after 24 hrs.)



Graph - 8: Antimicrobial effect on the *L. parasiticus* plants (after 48 hrs.)

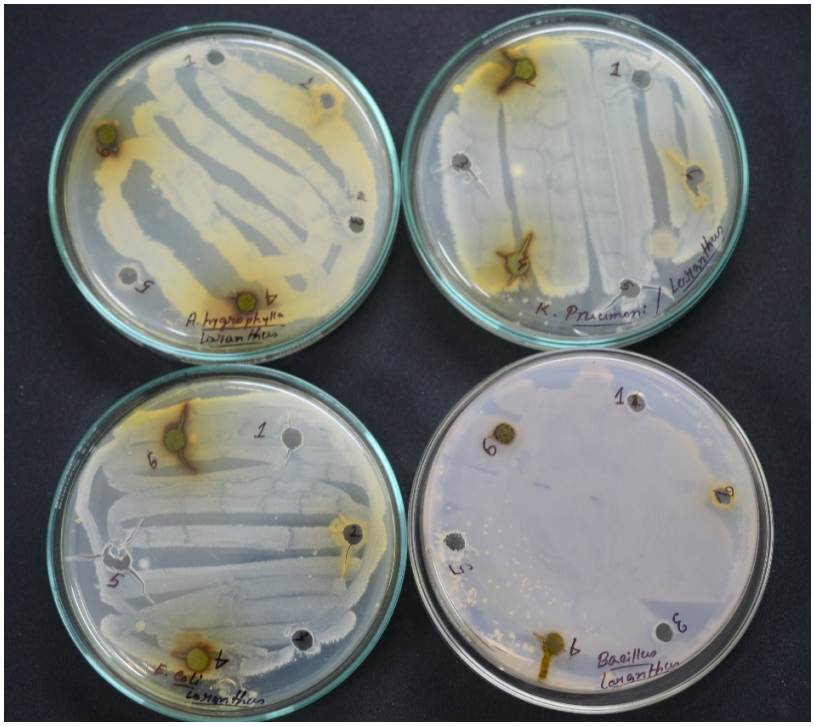


Plate- 1: Antimicrobial activity of *L. parasiticus* extract on all four bacteria.

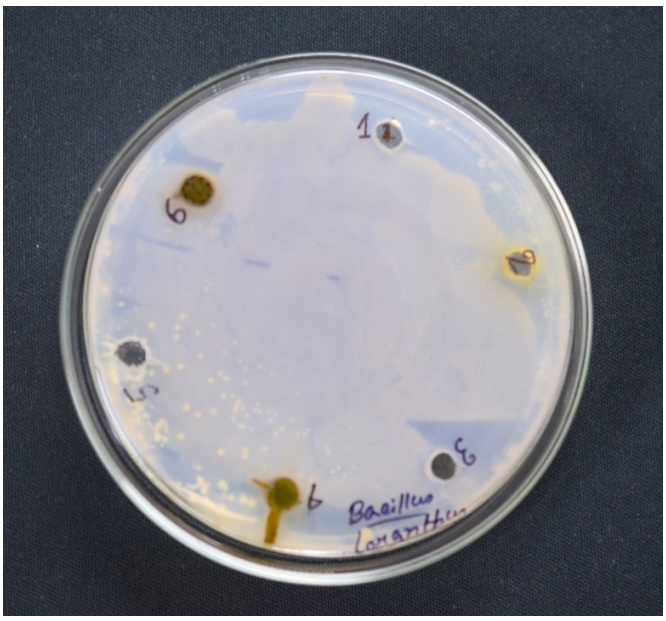


Plate- 2: Plant extract (Acetone, Aqueous, Methanol) which show antimicrobial activity on *Bacillus subtilis.*

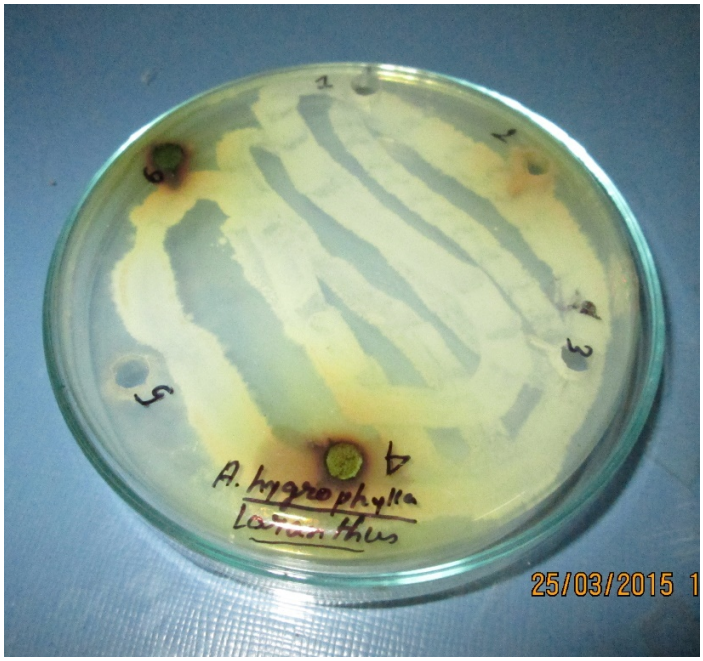


Plate- 3: Plant extract (Acetone, Aqueous, Methanol) which show antimicrobial activity on *Aeromonas hydrophila*

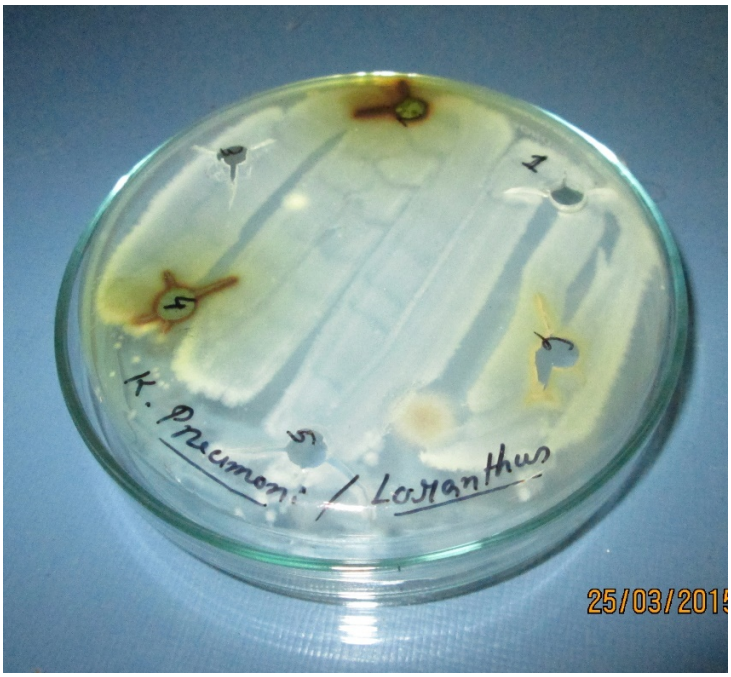


Plate- 4: Plant extract (Acetone, Aqueoous, Methanol) which showantimicrobial activity on *Klebsiella pneumoniae*.

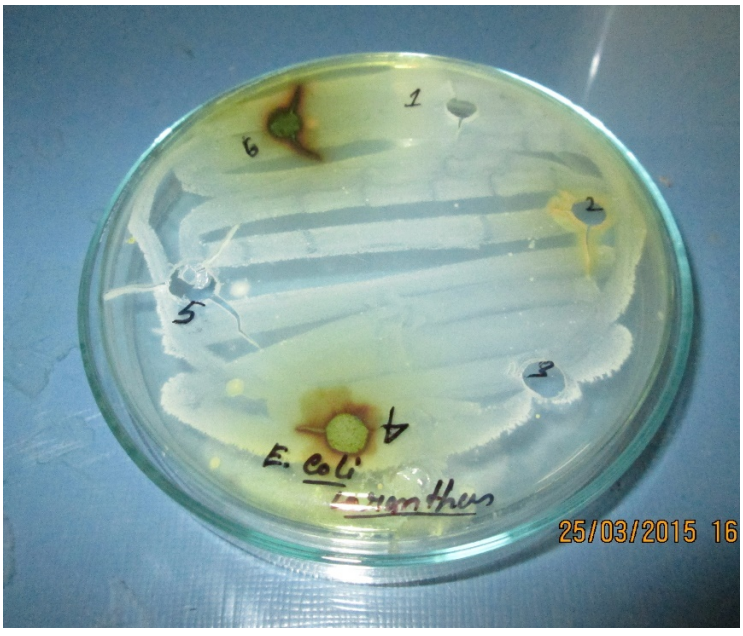


Plate- 5: Plant extract (Acetone, Aqueous, Methanol) which show antimicrobial activity on *Escherichia coli.*

X.A.1.ii - Discussions

Loranthus parasiticus exhibited certain degrees of antibacterial activity against different bacterial strain. *Loranthus parasiticus* with its aqueous extract of showed inhibition zone of 10.5 mm only against *K. pneumonia*, against *A. hydrophilla*, *E. coli*, *B. subtilis* is 10 mm, 9 mm, 9mm respectively. But Roy,et al; (2015), Preliminary phytochemical analysis with leaf extract of *Loranthus* with reference to their anti-microbial activity had been showed that there is no effect on *K.pneumonia* of plant aqueous extract. He showed that the Methanolic extract of *Loranthus parasiticus*inhibition zone of 2 mm against *Eschericheia coli*, and *Klebsiella pneumoniae* each and 5 mm against *Bacillus subtilis* after 24 hrs.incubation.The inhibition zone extended to 7mm in case of *B. subtilis* after 48 hrs which remained unchanged in case of others. But in our study, proved that the Methanolic extract of *L. parasiticus* inhibition zone of 12 mm against *E. coli* and *K. pneumonia* each, after 24 hrs. incubation. After 48 hrs. incubation the inhibition zone extended to 1.5mm in case of *A. hydrophila*, 2.5mm, 2mm, 2mm in case of *B. subtilis, K. pneumonia, E. coli* respectively. According to Roy, et al; (2015) Acetone extract of *Loranthusparasiticus* showed inhibition zones of 2 mm against*Eschericheia coli* and *Klebsiella pneumoniae*each and 5 mm against *Bacillus subtilis* after 24 hrs incubation. The inhibition zone extended to 7 mm for *B. subtilis* after 48 hrs. but a secondary inhibition zone was also observed in case of *E. coli and K.pneumoniae* after 48 hrs which extended to 13 mm and 8 mm respectively. But in our study after 48 hrs. incubation, the inhibition zone extended to 3mm in case of *K. pneumonia*, 2mm, 1mm in case of *B. subtilis* and *A.hydrophila* respectively, but in case of E. coli there has no change of inhibition zone.

X.A.2.i - Results (*Macrosolon cochichinensis*)

The result of screening plant extracts for antimicrobial activity was summarized in the (Table. 9, 10 and 11). It was found that the plant extract had certain degree of antimicrobial activity. Methanolic extract of *Macrosolon cochichinensis* showed inhibition zone of 10mm against *klebsella pneumonia* and 8mm against *Bacillus subtilis* and 7mm against *Aeromonas hydrophila*and *Eschericheia coli*after 24 hrs. incubation. The inhibition zone extended to 10mm for *Bacillus subtilis* and 8mm for *Eschericheia coli* and the inhibition zone remain unchanged in case of *klebsella pneumonia andAeromonas hydrophila* after48 hrs. incubation.

Acetone extract of *Macrosolon cochichinensis* showed inhibition zone of 14mm against *Bacillus subtilis*and and 8mm against *Aeromonas hydrophila* 5mm against *Eschericheia coli* and 4mm against *Klebsella pneumonia* after 24 hrs.incubation. The result remained unchanged after 48 hrs. incubation, except in case of *Eschericheia coli* the inhibition zone extended to 6mm.

Aqueous extract of *Macrosolon cochichinensis* showed inhibition zone of 4mm against *Bacillus subtilis* and *Eschericheia coli*, 3mm against *Klebsiella pneumonia and Aeromonas hydrophila* after 24 hrs incubation.The result remains unchanged after 48 hrs. incubation except in case of *Aeromonas hydrophila* where inhibition zone extended to 4mm.

Table - 25: Antimicrobial effect of Methanolic extracts of the *M. cochinchinensis*

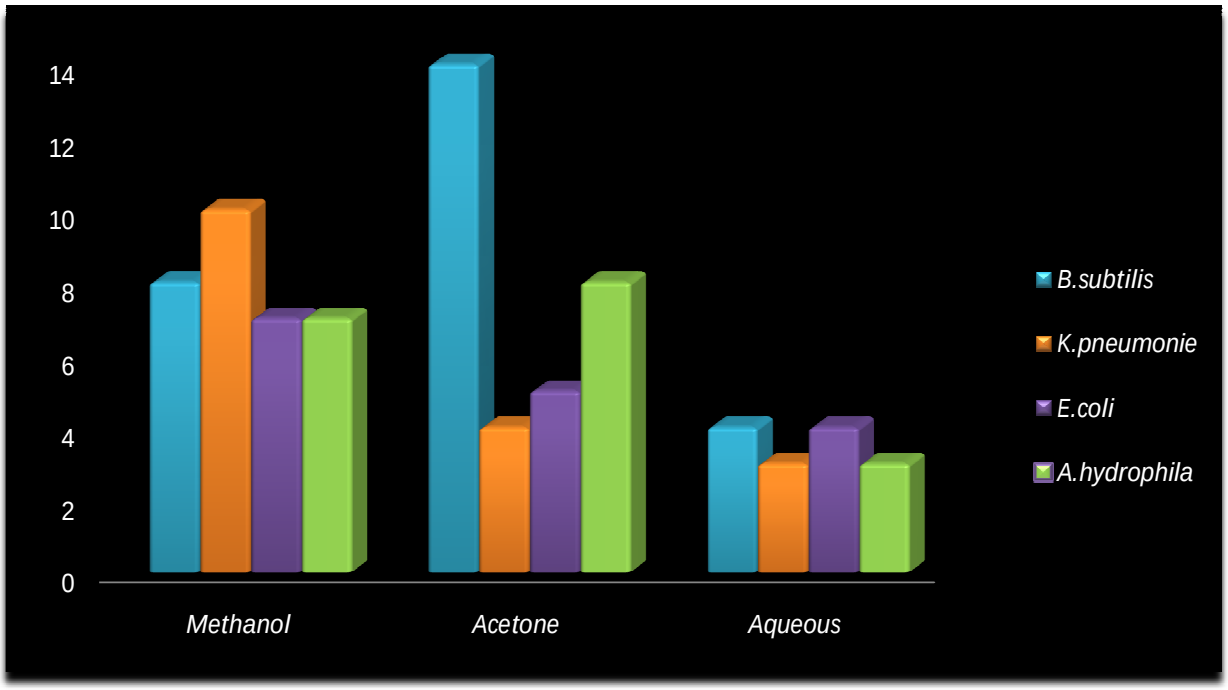
Plant	Diameter of the inhibition zone(mm)							
M. cochinchinensis	*Bacillus subtilis*		*Klebsiella pneumonia*		*Escherichia coli*		*Aeromonas hydrophila*	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	8	10	10	10	7	8	7	7

Table - 26: Antimicrobial effect of Acetone extracts of the *M. cochinchinensis*

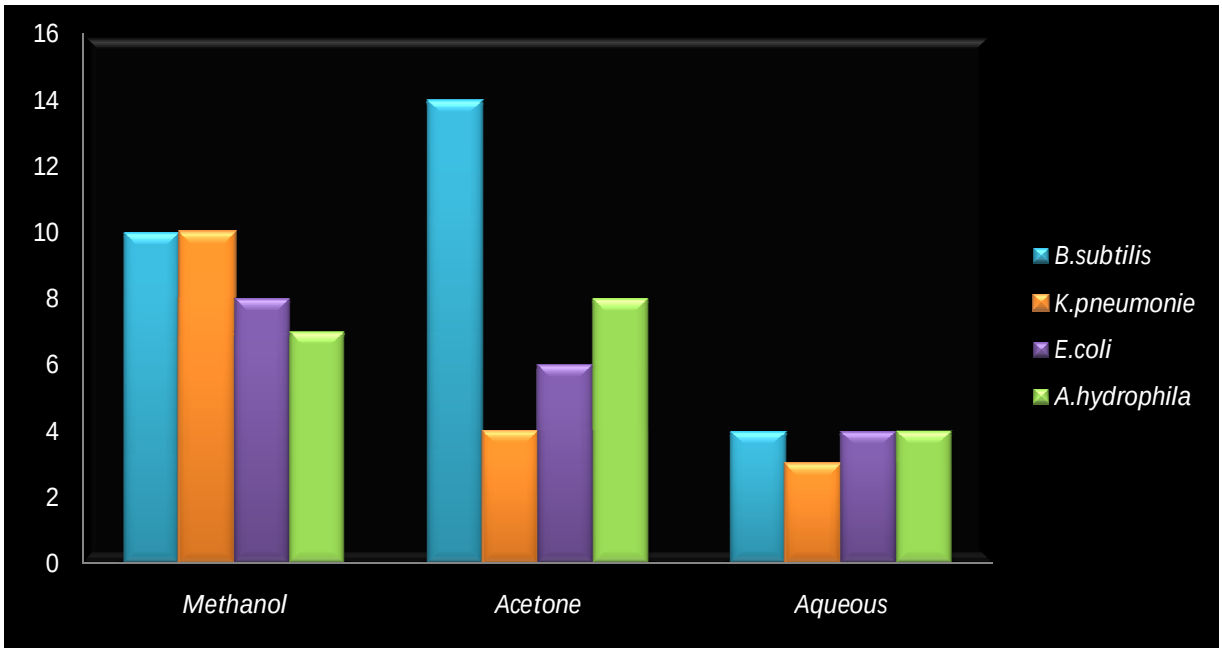
Plant	Diameter of the inhibition zone(mm)							
M. cochinchinensis	*Bacillus subtilis*		*Klebsiella pneumonia*		*Escherichia coli*		*Aeromonas hydrophila*	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	14	14	4	4	5	6	8	8

Table -27: Antimicrobial effect of Aqueous extracts of the *M. **cochinchinensis***

Plant	Diameter of the inhibition zone(mm)							
M. cochinchinensis	*Bacillus subtilis*		*Klebsiella pneumonia*		*Escherichia coli*		*Aeromonas hydrophila*	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	4	4	3	3	4	4	3	4



Graph - 9: Antimicrobial effect on the *M. cochinchinensis* plants (after 24hrs).



Graph - 10: Antimicrobial effect on the *M. cochinchinensis* plants (after 48hrs).

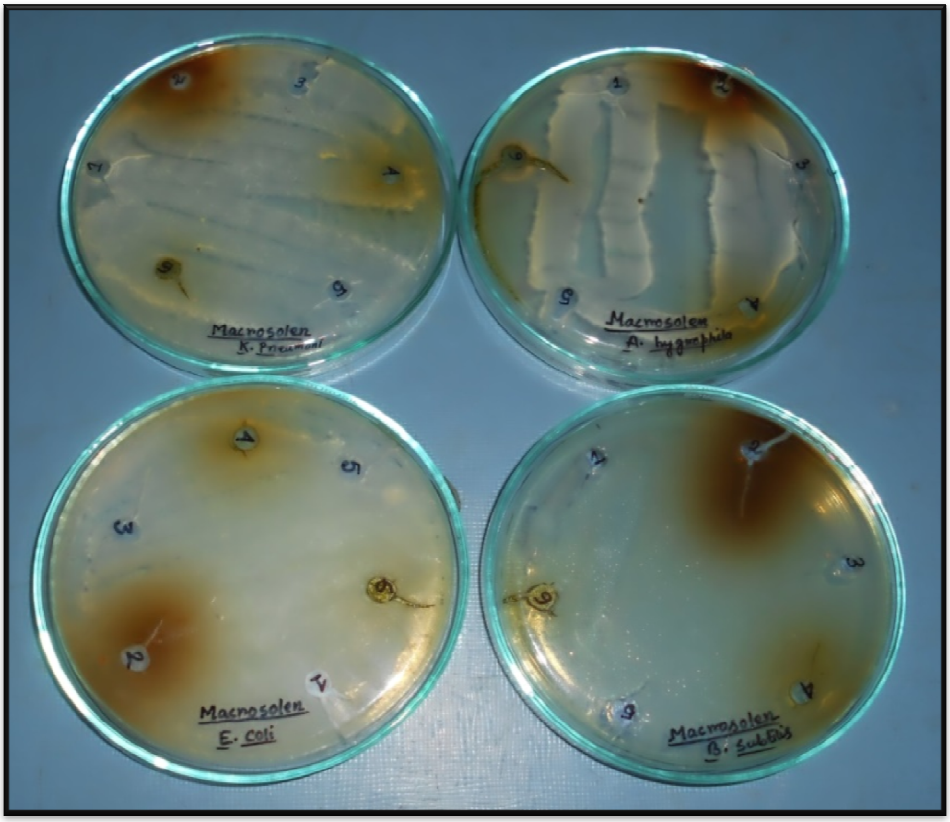


Plate- 6: Antimicrobial activity of *M.cochinchinensis* extract on all four bacteria.

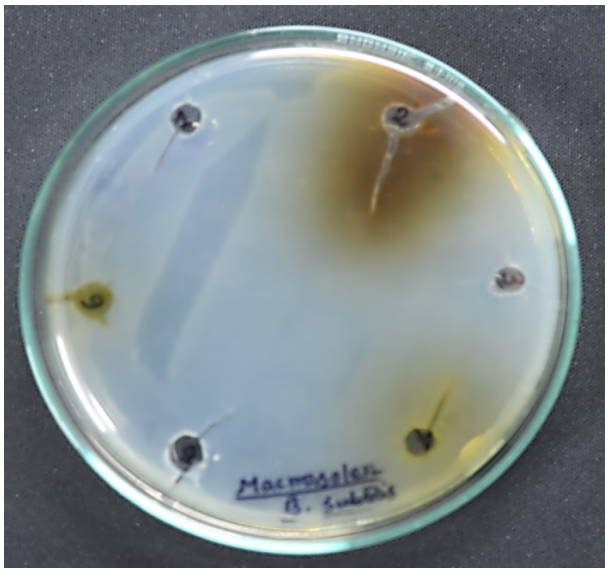


Plate- 7: Plant extract which shows antimicrobial activity on *Bacillus subtilis*.

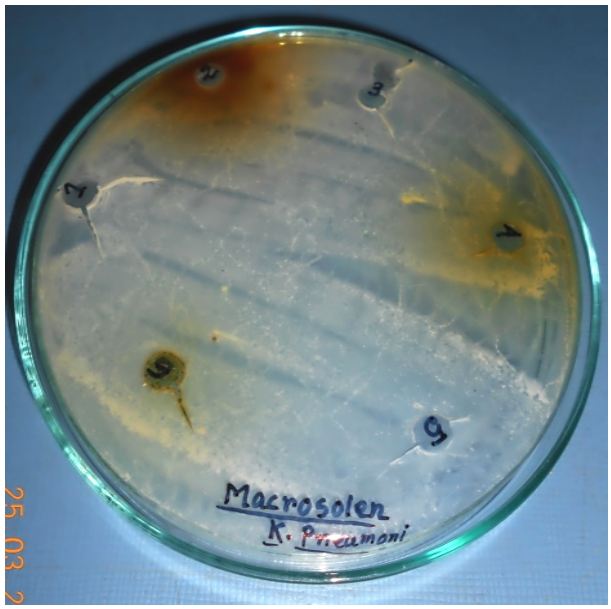


Plate- 8: Plant extract which shows antimicrobial activity on *Klebsiella pneumonia.*

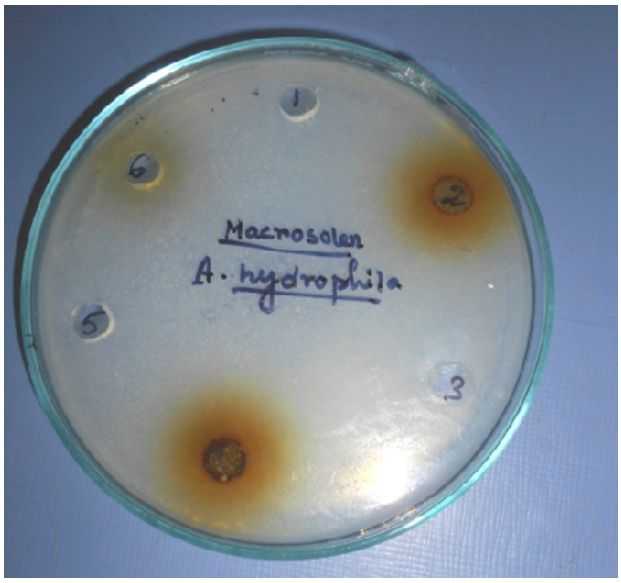


Plate- 9: Plant extract which shows atimicrobial activity on *Escherichia coli.*

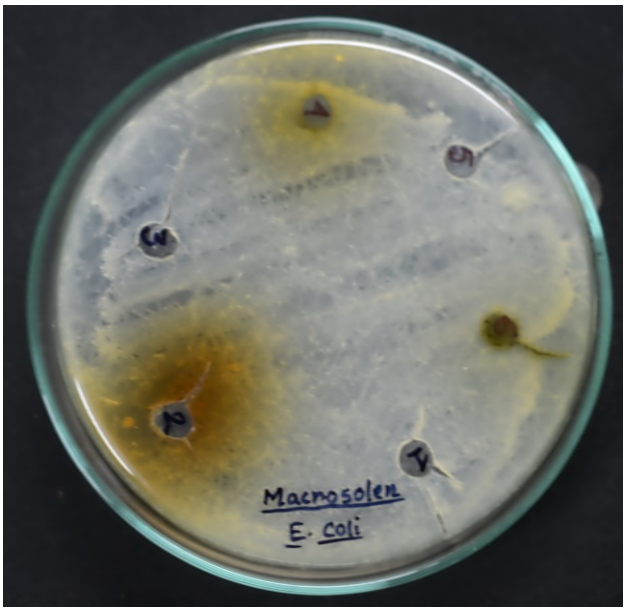


Plate- 10: Plant extract which shows antimicrobial activity on *Aeromonas*

X.A.1.ii - Discussion

The non-cytotoxic concentrations of plant extracts were used for antimicrobial activity tests. The aqueous extract showed less potent anti microbial activity with compared to other to extracts. In my study, *Macrosolen cochichinensis*, it was the acetone extraction which gave the best result in comparison to others. The plant extracts have the capacity to inhibit bacterial strains. The diameter of inhibition zone increase minutely from 24 hrs. to 48 hrs. and an interesting effect is found,after 48 hrs. a secondary inhibition zone formed around the prominent inhibition zone. From this investigation, it was found that the bacteria *Bacillus subtilis* highly inhibited by the methanolic solution of *Macrosolen cochinchinensis*. *Bacillus subtilis* is greatly inhibited by acetonic solution of *Macrosolon cochichinensis* here by it can be stated that *Macrosolen cochinchinensis* has the power to inhibit the bacterialcolony as it shows its effectiveness against all the bacterial strains.This investigation revealed that the bacterial inhibition can vary with the solvent usedfor

extraction, and the organisms tested. From this point of view, methanolic and acetonic solution is more effective than the other one. The plant extracts have the capacity to inhibit bacterial strains. Acetonic extract of *Macrosolen cochichinensis* is very much active against *Bacillus subtilis*.

X.A.3.i - RESULT (*Viscum album*)

The result of screening plant extracts for antimicrobial activity was summarized in the Table No. 1, 2& 3. It was found that the plant extract had antimicrobial activity of different degrees. **Aqueous** extract of *Viscum album shows*, after 24 hrs. incubation that *Viscum album* produced inhibition zone against *Bacillus subtilis* (2.0cm), *Klebsiellapneumoniae* (1.5cm) and Escherichia coli (1.8cm). But in case of *Aeromonas hydrophila* inhibition zone was (2.0cm). But in case of **Methanolic** extract of *Viscum album* show inhibition zone of *Bacillus subtilis*(2.0cm), *Klebsiellap neumoniae*(2.5cm), *Escherichia coli* (2.2cm), *Aeromonus hydrophila* (2.4cm), so in case of methanoic extract the inhibition zone is highest of *A. hydrophilla*. In case of **Acetone** extract the inhibition zone is highest of *A. hydrophilla* (2.6cm) and K. pneumonia (2.6cm). against other three bacterial strain, *Bacillus subtilis* (2.0cm), *Escherichia coli* (2.5cm).

After 48 hrs.Incubation it was found that the previously formed inhibition zones were increasedin all bacterial strain in case of **aqueous** extract. *Escherichia coli* (2.0cm), *Klebsiella pneumoniae* (1.8cm), *Bacillus subtilis* (2.3cm) &*Aeromonas hydrophila* (2.4cm). But in case of **Acetone** extract the inhibition zone are unchanged of *E. coli* and another strain are increase *K. pneumoniae*(3.3cm), *B. subtilis* (2.3cm), *A. hydrophilla*(2.8cm) and

E. coli(3.0cm). *K. pneumoniae*. (3.2cm), *B. subtilis* (2.3cm), *A. hydrophilla* (2.9cm) and *A. hygrophilla* (2.9cm) in case of **Methanol** extract of *Viscum album* plant.

Table - 28: Antimicrobial effect of the Methanolic extracts of *Viscum album*

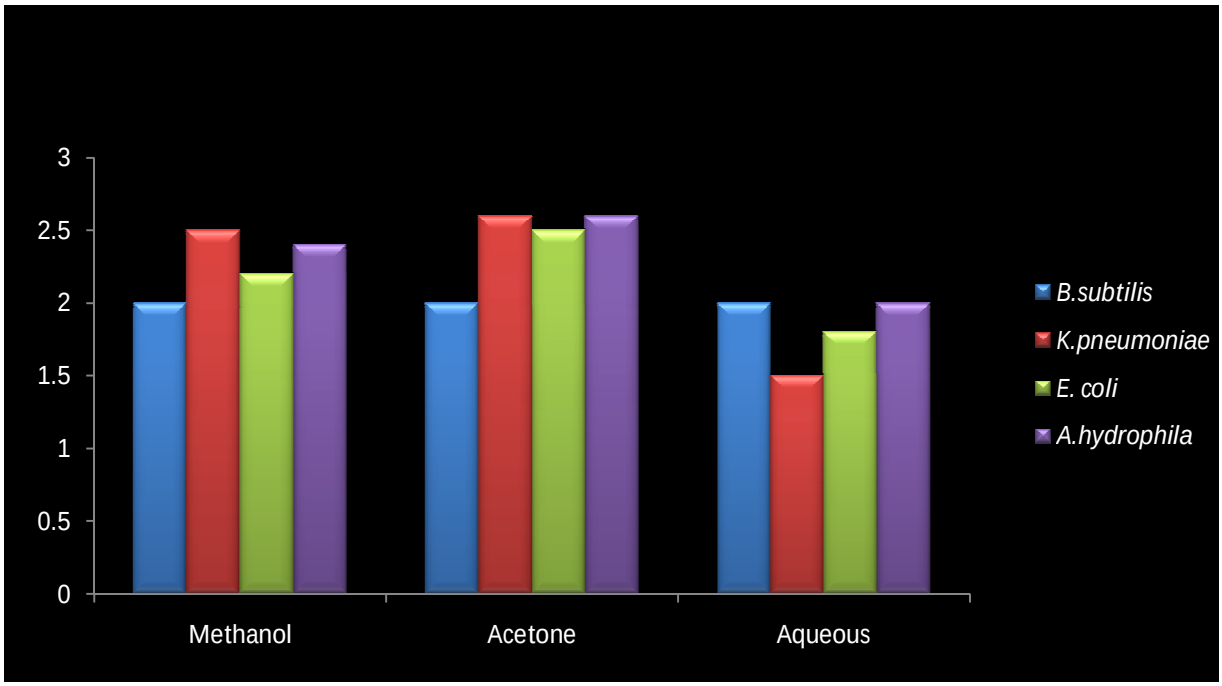
Plant	Diameter of the inhibition zone(mm)							
M. cochinchinensis	*Bacillus subtilis*		*Klebsiella pneumonia*		*Escherichia coli*		*Aeromonas hydrophila*	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	2.0	2.3	2.5	3.2	2.2	3.0	2.4	2.9

Table - 29: Antimicrobial effect of the Acetone extracts of *Viscum album*

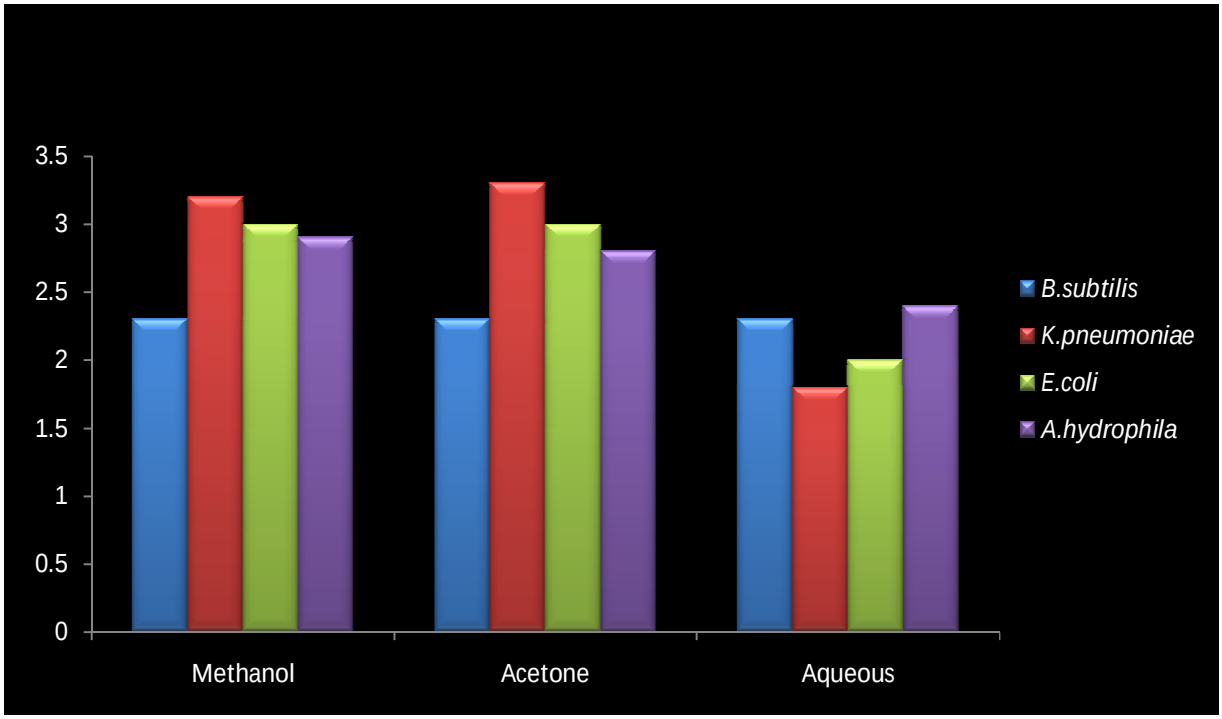
Plant	Diameter of the inhibition zone(mm)							
M. cochinchinensis	*Bacillus subtilis*		*Klebsiella pneumonia*		*Escherichia coli*		*Aeromonas hydrophila*	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	2.0	2.3	2.6	3.3	2.5	3.0	2.6	2.8

Table – 30: Antimicrobial effect of the Aqueous extracts of *Viscum album*

Plant	Diameter of the inhibition zone(mm)							
M. cochinchinensis	*Bacillus subtilis*		*Klebsiella pneumonia*		*Escherichia coli*		*Aeromonas hydrophila*	
	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs	24hrs	48hrs
	2.0	2.3	1.5	1.8	1.8	2.0	2.0	2.4



Graph – 11: Antimicrobial effect on the *V.album* plant after 24 hrs



Graph - 12: Antimicrobial effect on the *V.album* plant after 48hrs

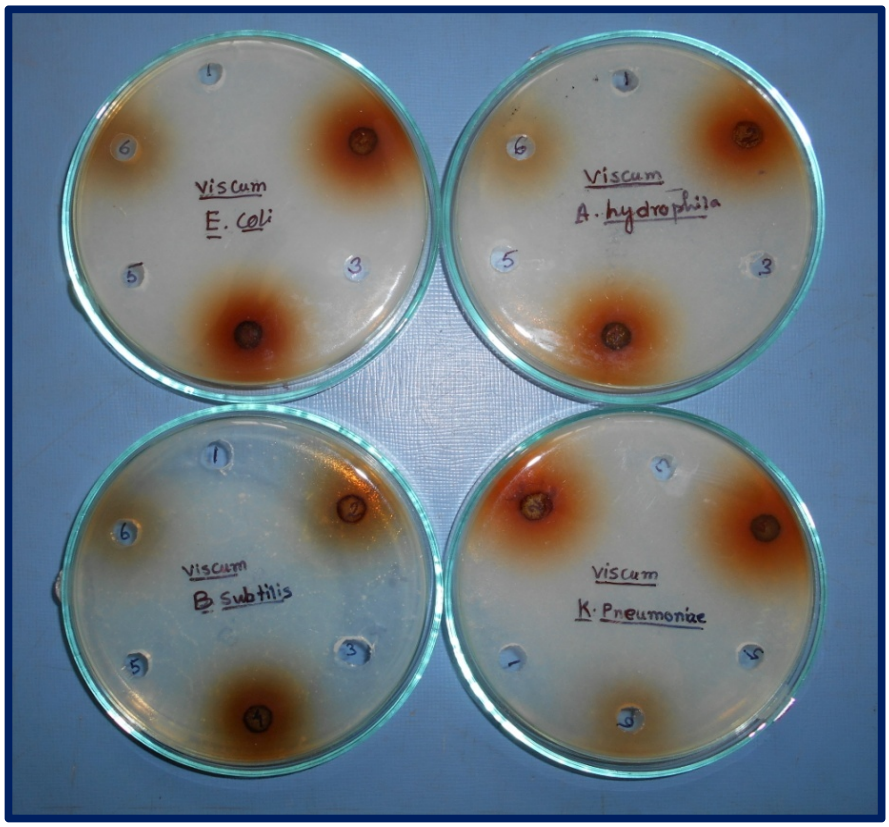


Plate - 11: Antimicrobial activity of *Viscum album* extract on all four bacteria

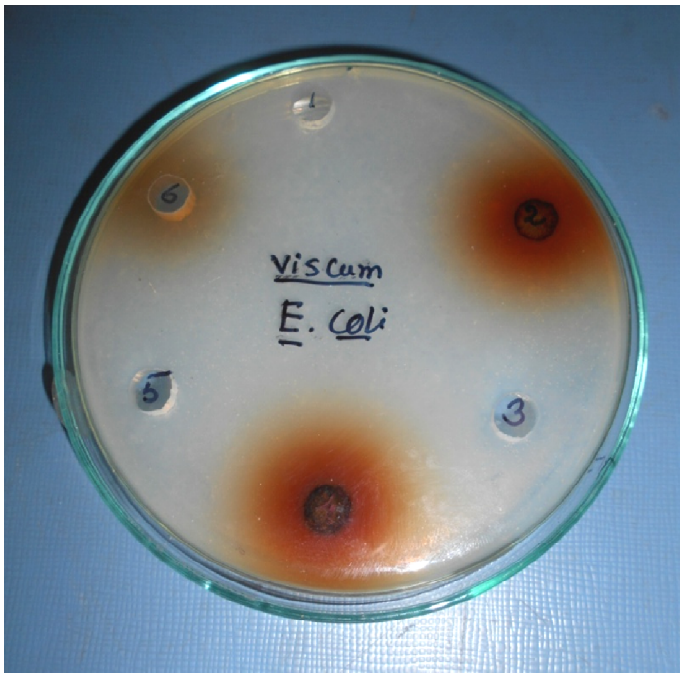


Plate – 12: Antimicrobial activity of *Viscum album* against *E. coli*

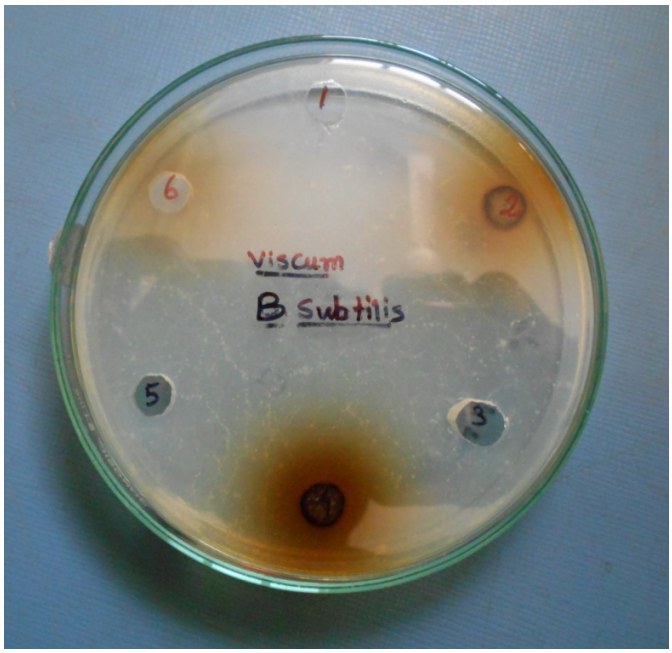


Plate – 13: Antimicrobial activity of *Viscum album* against *Bacillus*

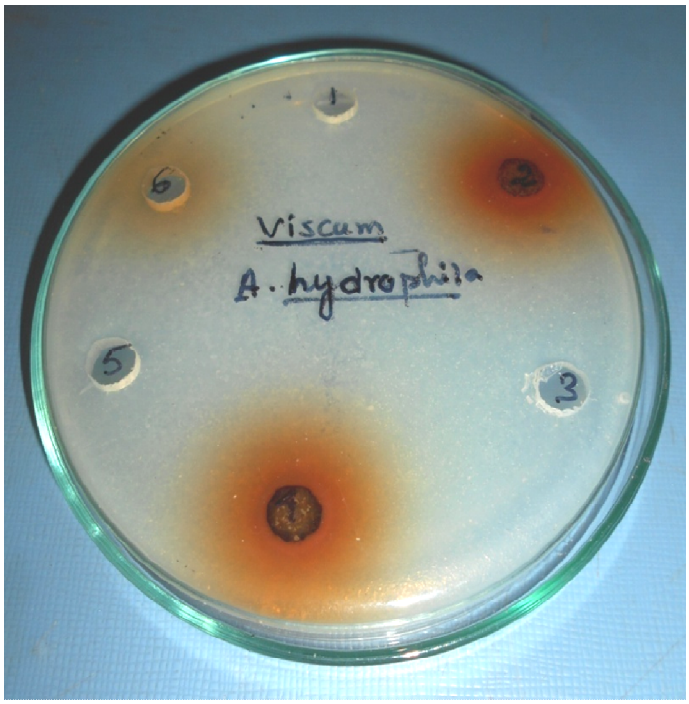


Plate – 14: Antimicrobial activity of *Viscum album* against *A hydrophillus*

Plate – 15: Antimicrobial activity of *Viscum album* against *K. pneumoniae*

X.A.3.ii - Discussion

The result of screening plant extracts for antimicrobial activity was summarized in the Table. It was found that the plant extract had antimicrobial activity of different degrees. **Aqueous** extract of *Viscum album* shows, after 24 hrs. incubation that *Viscum album* produced inhibition zone against *Bacillus subtilis* (2.0cm), *Klebsiella pneumoniae* (1.5cm) and Escherichia coli (1.8cm). But in case of *Aeromonashydrophila* inhibition zone was (2.0cm). But in case of **Methanolic** extract of *Viscum album* show inhibition zone of *Bacillussubtilis*(2.0cm), *Klebsiella pneumonia* (2.5cm), *Escherichia coli* (2.2cm), *Aeromonus hydrophila* (2.4 cm), so in case of methanoic extract the inhibition zone is highest of *A. hydrophilla*. In case of **Acetone** extract the inhibition zone is highest of *A. hydrophilla* (2.6cm) and K. pneumonia (2.6cm). against other three bacterial strain, *Bacillus subtilis* (2.0cm), *Escherichia coli* (2.5cm).

After 48 hrs.Incubation it was found that the previously formed inhibition zones were increasedin all bacterial strain in case of **aqueous** extract. *Escherichia coli* (2.0cm), *Klebsiella pneumoniae* (1.8cm), *Bacillus subtilis* (2.3cm) & *Aeromonas hydrophila* (2.4cm). But in case of **Acetone** extract the inhibition zone are unchanged of *E. coli* and another strain are increase *K. pneumoniae*(3.3cm), *B. subtilis* (2.3cm), *A. hydrophilla* (2.8cm) and *E. coli* (3.0cm). *K. pneumoniae*. (3.2cm), *B. subtilis* (2.3cm), *A. hydrophilla* (2.9cm) and *A. hygrophilla* (2.9cm) in case of **Methanol** extract of *Viscum album* plant.