Final Report of U G C Minor

Research Project on

Phytochemical and Antimicrobial studies of

Abrus precatorius L

By

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Introduction:

Rosary pea is a wonderful herb. A small climbing tropical vine with seeds known as crab's eye. This herb is having much importance in a common tribal life. They make various ornaments by the seeds at their festive time, press bird's feather and Gunja seeds on beeswax and tie it on their waist. Seeds of the plants are having reputation as one of the world's most deadly seeds. Precatory beans are certainly one of the most beautiful seeds on earth. Seeds have remarkably uniform weight of 1/10th of a gram. Seeds were used by goldsmiths as standard weights for weighing gold and silver in previous time. This plant species is propagated through seeds.

Common names in World: Rosary pea; Blackeyed Susan, Crab's eye, Jequerity, Pois rouge, Precatory bean, Tento muido, Tento muido, Cain Ghe, Graines Reglisse, Gunchi, Hint Meyankoku, Hung Tou, Jequerit, Liane Reglisse, Ma Liao Tou, Paratella, Paternoster, Peonia De St Tomas, Peonia, Pois Rouge, Reglisse, To-Azuki, Weesboontje, Rakat.

Local names in India: Gunj (Marathi) ;Chunhali, Kunch (**Bengali**); Chanothi, Gunja (**Gujarati**); Ganchi, Gunchi, Rati (**Hindi**); Ganji, Gul-Ganju, Guluganji, Madhuka (**Karnataka**); Kunni, Kunnikuru (**Malayalam**); Chanoti, Gunchi, Gunja (**Marathi**); Gundumani, Kuntumani (**Tamil**); Guruginia, Guruvenda (**Telugu**); Gunja, Runji (**Oriya**) Liluwani, Raturmani (**Assam**); Labrigunchi, Ratak(**Punjab**).

Taxonomic description: A beautiful, much-branched, slender, perennial, deciduous, woody, prickly twining or climbing herb. Stem cylindrical, wrinkled; bark smooth-textured, brown. Leaves stipulate, pinnately compound; leaflets 7-24 pairs, turgid, oblong, obtuse, truncate at both ends, appressed hairy. Flowers in axillary racemes, shorter than leaves, fascicled on the swollen

nodes, pink or pinkish-white; calyx-lobes short, appressed hairy. Pods turgid, oblong, with a sharp deflexed beak, silky-textured, 3 to 5-seeded. Seeds elliptic to sub-globose, smooth, glossy, shining red with black blotch around the hilum.

Plate I: Entire plant of *Abrus Precatorius*

Plate II: Inflorescence and pods of *Abrus Precatorius*

Plate III: Flower of *Abrus Precatorius*

Plate IV: Fruits of *Abrus Precatorius*

Plate V: Plant of *Abrus Precatorius* as climber.

Plate VI: Seeds of *Abrus Precatorius*

Plate VII: Necklace of Seeds of *Abrus Precatorius*

Plate VIII: Enlareged Seeds .

Habitat : It is a common plant in Kwazulu-Natal and Limpopo Province and native to many tropical areas of the world. A common weed of roadsides, old gardens, disturbed sites, waste areas and waterways in the higher rainfall regions of subtropical areas. It has also been reported from open woodlands, rainforest margins and coastal dunes. *Abrus precatorius* reproduces mainly by seed. These seeds are thought to be mostly bird-dispersed, but it is likely that they are also spread along waterways during floods and in dumped garden waste. Commonly found as twining herb in mixed deciduous forests, in moist shady localities, grows best in fairly dry regions at low elevations.

Distribution: It is native to India, introduced to warmer regions of the world. It is indigenously found throughout India, even at altitudes up to 1200m on the outer Himalayas. It is now naturalized in all tropical countries. It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, North America, Tropical Africa and the West Indies. It also grows in all tropical or subtropical areas. It is used as an ornamental throughout North America.

Medicinal Importance: The seeds are considered abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, emollient, febrifuge, hemostat, laxative, purgative, refrigerant, sedative, vermifuge, antidote and used in various ailments to cure headache, snakebite, blennorrhagia, boil, cancer, cold, colic, conjunctivitis, convulsion, cough, diarrhea, fever, gastritis, gonorrhea, jaundice, malaria, night-blindness, ophthalmia and rheumatism. The seeds are also used to treat diabetes and chronic nephritis.

Leaves, roots and seeds are used for medicinal purposes. The plant is used in some traditional medicine to treat scratches and sores and wounds caused by dogs, cats and mice, and is also used with other ingredients to treat leucoderma. The leaves are used for their anti-suppurative properties. They are ground with lime and applied on acne sores, boils and abscesses. The plant is also traditionally used to treat tetanus, and to prevent rabies. Various African tribes use powdered seeds as oral contraceptives.

Paste of roots is administered to cure abdominal pains and tumors. The paste with fresh rhizome of 'Haldi' *(Curcuma longa)* is applied on wounds. This paste is also taken orally as a single dose once only for abortion. Grinded roots of *Abrus precatorius* is taken with pure clarified butter thrice a day for four days to cure cough. For graying of hair, a paste of leaves and seeds is made and juice is extracted. This juice is applied on hair as oil once a day one hour before taking bath.

Dry seeds of *Abrus precatorius* are powdered and taken one teaspoonful once a day for two days to cure worm infection.In veterinary medicine, it is used in the treatment of fractures. Roots of *Abrus precatorius,* 3-5 black pepper and 5 g of dried ginger are mixed and the paste is given orally to get rid of evil spirits or black magic. A piece of root is also tied on the arm to get immediate result.It is an ingredient of product "Tranquil" used in the treatment of stress and anxiety.

Uses : The plant secretes the toxin called abrin which is closely related to ricin. The symptoms of these toxins are severe stomach pains diarrhoea, nausea, cold sweat, drowsiness, colic and weak and fast pulse. The seeds of *Abrus precatorius* are valued in making necklaces for their bright coloration. They are sold in stores and distributed throughout the world by travelers. They are favourite playing toys for children. The seeds are considered abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, emollient, febrifuge, hemostat, laxative, purgative, refrigerant, sedative, vermifuge, antidote and used in various ailments to cure headache, snakebite, blennorrhagia, boil, cancer, cold, colic, conjunctivitis, convulsion, cough, diarrhea, fever, gastritis, gonorrhea, jaundice, malaria, night-blindness, ophthalmia and rheumatism. The seeds are also used to treat diabetes and chronic nephritis.

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Other Uses: The brightly-coloured seeds attract childrens; they also play with them and in school use them in their handiwork and to count. Necklaces and other ornaments are made from

the seeds and worn by both children and adults. Leaves and seeds are nutritious. Boiled seeds are eaten in certain parts of India. It is claimed that cooking destroys the poison of seeds. The small seeds are used in jewelry. They have a uniform weight of 1/10th of a gram, hence used as weighing unit. Seeds have also the potential of good insecticide and antimicrobial activity.

Chemical Components: Seeds are poisonous and contain abrin, a poisonous principle. The sweet principle glycyrrhizin is same as that of liquorice.Abrine, Abraline, Abrasine, Abricin, Abrin, Abrusgenic-acid, Abrusgenic-acid-methyl-ester, Abruslactone, Abrussic-acid, Anthocyanins, Ash, Calcium, Campesterol, Choline, Cycloartenol, Delphinidin, Gallic-acid,, Glycyrrhizin, Hypaphorine, N,n-dimethyl-tryptophan, N,n-dimethyl-tryptophan-metho-cation-methyl-ester, P-coumaroylgalloyl-glucodelphinidin, Pectin, Pentosans, Phosphorus, Delphinidin, Gallic-acid,, Glycyrrhizin, Hypaphorine, N,n-dimethyl-tryptophan, N,n-dimethyl-tryptophan-metho-cation-methyl-ester, P-coumaroylgalloyl-glucodelphinidin, Pectin, Pentosans, Phosphorus, Picatorine, Polygalacturonic-acids, Precasine, Precatorine and Protein Trigonelline determined in the plant.

Toxic effect: The seeds are highly toxic due to presence of Abrin, a protein. It may be fatal if eaten. The primary symptoms include nausea, vomiting, severe abdominal pain and diarrhea, burning in throat; later ulcerative lesions of mouth and esophagus.

Ingested seeds can affect the gastrointestinal tract, the liver, spleen, kidney, and the lymphatic system. Infusion of seed extracts can cause eye damage, conjunctivitis and even blindness after contact. The major symptoms of poisoning are acute gastroenteritis with nausea, vomiting and diarrhoea leading to dehydration, convulsions, and shock. Dehydration, as well as direct toxicity on the kidneys, could result in oliguria that might progress to death in uraemia.

Abrin, which consists of abrus agglutinin, and toxic lectins abrins a, b, c and d are the five toxic glycoproteins found in the seeds. Abrin is a ribosome - inactivating protein which blocks protein synthesis and is one of the most deadly plant toxins known. The toxin is released only after broking of seeds.

Materials and Methods:

Phytochemical Studies : Collection of sample

The Plants were collected from the field and chopped into small pieces, shade dried and coarsely powdered.

Preparation of extracts:

The powdered sample obtained was subjected to successive extraction with methanol and distill water by soxhlet method. The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of methanol solvent was removed. The resulted extracts were used for preliminary phytochemical screening.

Test for alkaloids: Extract was dissolved individually in dilute Hydrochloric acid and Solution was clarified by filtration.

a. Mayer's Test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b. Wagner's Test: Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c. Dragondroffs Test: Filtrate was treated with Dragondroffs reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Test for Flavonoids:

a. Alkaline Reagent Test: The Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl acid, indicates the presence of flavonoids.

b. Lead acetate Test: The Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Test for Carbohydrate:

a. Molisch's Test: To 1 ml of extract, 2 drops of Molisch's regent was added in a test tube and 2 ml of concentrate H_2SO_4 was added carefully keeping the test tube slightly curved. Formation of violet ring at the junction indicated the presence of carbohydrate.

b.Benedict's Test: Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

c. Fehling's Test: Extracts were hydrolyzed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of carbohydrate.

Test for phenols:

a. Ferric Chloride Test: The aqueous solution of extract was treated with three drops of freshly prepared 1% ferric chloride and potassium ferrocyanide. Formation of bluish-green colour was taken as positive. The methanol extract was dissolved in water. Few crystals of ferric sulphate were added to the mixture. Formation of dark violet colour indicated the presence of phenolic compounds.

Test for saponins

a. Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

Test for Proteins:

a.Biuret Test: Test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color.

Test for phytosterols

a. Salkowski's test: The extract was dissolved in 2 ml chloroform in a test tube. Conc. Sulphuric acid was carefully added on the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicated the presence of a steroid ring i.e., glycoside.

b. Liebermann Burchard's test: The Extract was treated with chloroform and filtered. The filtrate was treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

Test for Oil & Fats:

a. **Filter paper test:** Small quantities of various extracts were pressed separately between the filter papers. Appearance of oil stain on the paper indicates the presence of fixed oils.

Test for Glycosides:

a. **Keller Killiani Test:** Test solution was treated with few drops of glacial acetic acid and Ferric chloride solution and mixed. Concentrated sulphuric acid was added, and observed for the formation of two layers. Lower reddish brown layer and upper acetic acid layer which turns bluish green would indicate a positive test for glycosides.

b. Borntrager's Test: To the 3ml of aqueous extract, dil. H2SO4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Test for Tannins

a. Ferric chloride test. The extract was dissolved in water. The solution was clarified by filtration; 10% ferric chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

b. Lead acetate test. The extract was dissolved in water and to that 10% Lead acetate solution was added. The appearance of yellow precipitate confirms the tannins.

In vitro Antimicrobial Activity : *Abrus precatorius* seeds were collected from field. The seeds were dried under shade and then powdered with a mechanical grinder and stored in an airtight container. The powder obtained was subjected to successive soxhlet extraction with the organic solvents. Bacterial Strains for in vitro antimicrobial activity was examined for aqueous and methanol extracts were *Enterococcus faecalis, Escherichia coli, Micrococus luteus, Lactobacillus fermentum*, *Klebsilla pneumonia, Staphylococcus aureus, Streptococcus thermophilus, Streptococcus mitis*, *Streptococcus mutans* and *Pseudomonas aeruginosa.* All the microorganisms were maintained on nutrient agar slants. The antimicrobial assay was performed by agar well diffusion method. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum and poured into the Petri plate. For agar disc diffusion method, the disc as saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. For agar well diffusion method, a well was prepared in the plates with the help of a cork-borer. 100µl of the test compound was introduced into the well. The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each bacterial strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented.

Results and Discussion: Phytochemical Studies :

Table: Phytochemical analysis of leaves of *Abrus precatorius*

S. No.	Phytoconstituents	Chemical Tests	Methanolic extract	Aqueous extract
1.	Alkaloids	Mayer's Test	-	-
		Wagner's Test	-	-
		Dragendorff's Test	-	-
2.	Flavonoids	Alkaline Reagent Test	+	+
		Lead acetate Test	+	+
3.	Carbohydrates	Molisch's test	+	+
		Barfoed's test	-	-
4.	Phenols	Ferric Chloride Test	+	+
5.	Saponins	Foam Test	-	-
6.	Proteins	Biuret Test	-	-
7.	Phytosterols	Salkowski's test	+	+
		Liebermann Burchard's test	+	+
8.	Oil & Fats	Filter paper test	-	-
9.	Glycosides	Keller Killiani Test	-	-
		Borntrager's Test	-	-
10.	Tannins	Ferric chloride test	+	+
		Lead acetate test	+	+

2)Antimicrobial studies :

1) **Immunstimulatory effects :** Abrin B derived from seeds of *A. precatorius* causes a strong agglutination of cells.

- This agglutination increases according to the order of differentiation in cells.
- .A non toxic dose of abrin (1,25µgram/kg body weight) can potentiate an immunresponse of a host, like increase in total leucocytes, weights of thymus and spleen.
- **2)Antifertility activity** : A methanol extract of A precatorius seeds impaired the motility of washed human spermatozoa with an EC 50 of 2.29 mg/ml, irreversibly. The highest concentration tested (20.0 mg/ml), the onset of the motility was almost immediate.
- The ethanol extract of leaves inhibits muscle preparations, like toad *Rectus abdominis* and rat diaphragm. The effects were reversible and depending on the contraction
- Native and heat - denaturized agglutinin from seeds of *A.precatorius* alters the macrophage function of mice in ***vitro***. Both substances are immune stimulants

- In an antifertility program with pregnant rats, *Abrus precatorius* was most effective . A daily dose of 3.30 or 300mg showed 40-60 percent inhibition of pregnancy of rats.
- This treatment met the energy metabolism of the *Cauda epididymidis*.
- Levels of acid phosphatase and succinic hydrogenase were significantly depleted, while protein and sialic acid appeared normal.

4) <u>Traditional uses</u> :

In the Ayurvedic medicine leaves of *Abrus precatorius* are laxative, expectorant and aphrodisiac medicines.

- Seeds are purgative, emetic, tonic, antiphlogistic, aphrodisiac and antiopthalmic.
- For the indigenous people they are potent phytomedicines, many of them in mixtures with other plants. Their toxicity is underestimated.
- In Asia beans are used as weights and jewellery is made from them by drilling.
- In Tanzania traditional healers claim the competence in the treatment of epilepsy. *A. precatorius* found between 60 plants commonly used against this illness.
- In Zimbabwe extracts of 58 plants popularly known to be effective against schistosomiasis were tested in vitro against excysted cysticercoids. Extracts of stem and root of *A.precatorius* were under the ten most effective samples.
- In the Indian Central Drug Research Institute at Lucknow discussions about an antifertility program are going on. One of the plants with priority is A.precatorius because of its ìestrogenicityî, nor because of its lectins.

5) Pharmacological effects :

- Abrin is toxic not only in normal animal cells but highly toxic against tumor cells too.
- Abrin suppresses the growth of solid Ehrlich ascites tumours, and fibrosarcoma in
- In cultured human cell lines, derived from acute lymphoblastic leukemia (ALL, Jurkat, CCRF-CEM, MOLT-4, and HPB-ALL) abrin-A induces apoptosis.
- This process was dose and time-dependent. It starts 1 hour after abrin-A application.
- Its maximum is on the third or fourth hour and ends with DNA-fragmentation on the fourth or sixth hour, depending on the cell line used.
- There was a positive correlation between the agglutinating activity of abrin-A, and the development of apoptosis till DNA fragmentation, finally.

- This suggests that the B-chain probably triggers the apoptosis,while the A-chain and breakage of the disulfide bond are responsible for its progress.

6) General applications:

- The seeds are considered abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, expectorant, emollient, febrifuge, hemostat, laxative, purgative, refrigerant, sedative, vermifuge, antidote and used in various ailments to cure headache, snakebite, blennorrhagia, boil, cancer, cold, colic, conjunctivitis, convulsion, cough, diarrhea, fever, gastritis, gonorrhea, jaundice, malaria, night-blindness, ophthalmia and rheumatism.
- The seeds are also used to treat diabetes and chronic nephritis.
- Leaves, roots and seeds are used for medicinal purposes. The plant is used in some traditional medicine to treat scratches and sores and wounds caused by dogs, cats and mice, and are also used with other ingredients to treat leucoderma.
- The leaves are used for their anti-suppurative properties. They are ground with lime and applied on acne sores, boils and abscesses.
- The plant is also traditionally used to treat tetanus, and to prevent rabies. Various African tribes use powdered seeds as oral contraceptives.
- Paste of roots is administered to cure abdominal pains and tumors. This paste is also taken orally as a single dose once only for abortion.
- Grinded roots of *Abrus precatorius* are taken with pure clarified butter thrice a day for four days to cure cough. For graying of hair, a paste of leaves and seeds is made and juice is extracted. It is applied on hair as oil once a day one hour before taking bath.
- Dry seeds of *Abrus precatorius* are powdered and taken one teaspoonful once a day for two days to cure worm infection.
- In veterinary medicine, it is used in the treatment of fractures.
- The seeds of *Abrus precatorius* are valued in making necklaces for their bright coloration. They are sold in stores and distributed throughout the world by travelers.
- They are favorite playing toys for children.
- The Seeds of *Abrus precatorius* very similar in weight. In older times Indians used to measure using these seeds and the measure was called as *Ratti*. This was used generally to measure gold and 1 Tola (11.6 Grams) = 12 Masha; 1 Masha = 8 Ratti.

***In vitro* Antimicrobial Activity :**

Sr.No.	Bacterial Strain	Zone of Inhibition (mm)
1	*Enterococcus faecalis*	11
2	*Escherichia coli*,	10
3	*Klebsilla pneumonia*	17
4	*Lactobacillus ermentum*	11
5	*Micrococus luteus*	12
6	*Pseudomonas aeruginosa*	09
7	*Staphylococcus aureus*	13
8	*Streptococcus mitis*	12
9	*Streptococcus mutans*	10
10	*Streptococcus thermophilus*	10

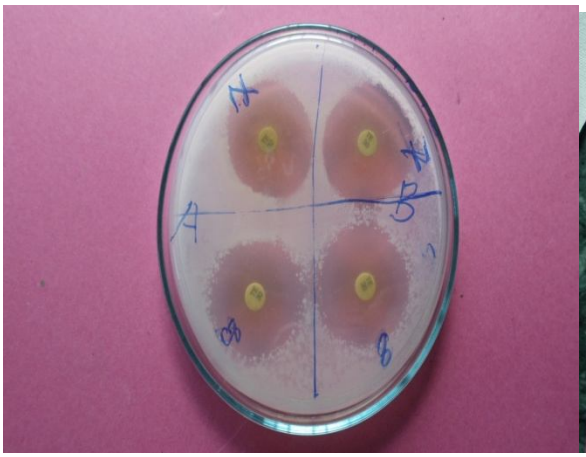

Plate-I: *Enterococcus faecalis*

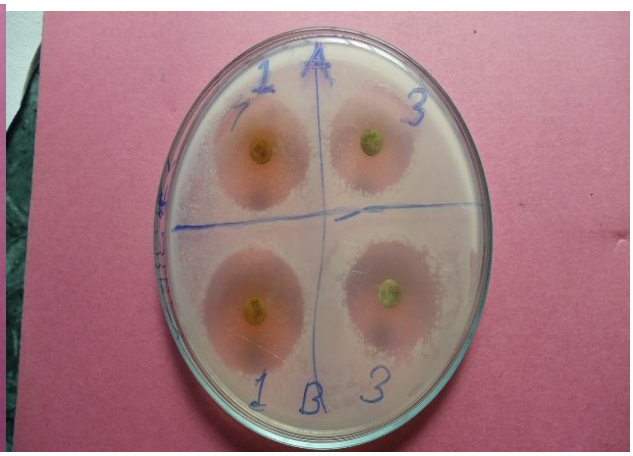

Plate-II: *Enterococcus faecalis*

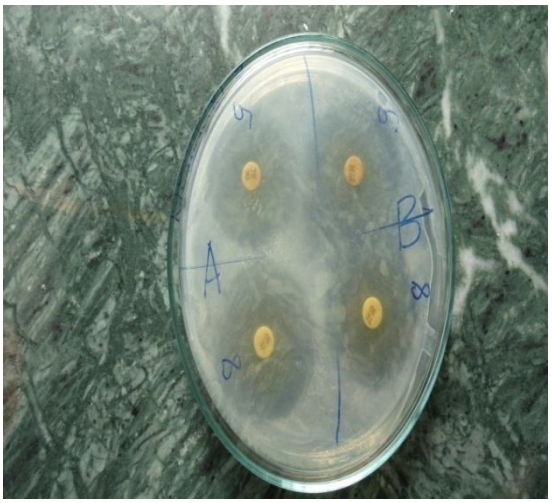

Plate-III : *Escherichia coli*,

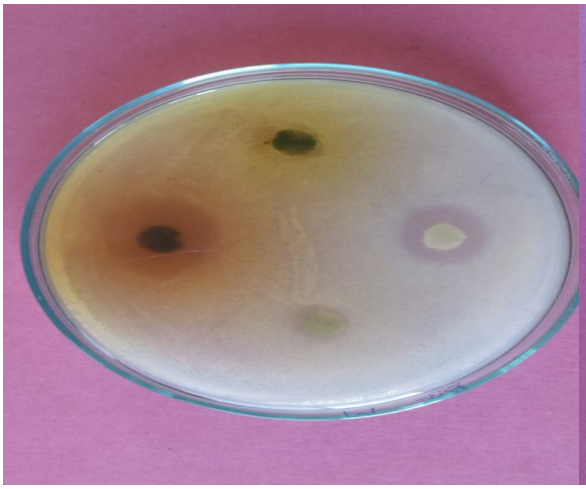

Plate-IV : *Lactobacillus ermentum*

Plate-V : *Lactobacillus ermentum*

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