



EVALUATION OF RICINIUS COMMUNIS EXTRACT FOR THE TREATMENT JAUNDICE.

MRS. SHUBHADA A KULKARNI (k.shubhada18@gmail.com) Assistant Professor

SYIPR

RADHA WATTAMWAR

SAYALI WAGH

Shekhar Jadhav

Abhishek Zaware

DR. GANESH G TAPDIYA, PRINCIPAL OF SYIPER

ABSTRACT – The Ricinus communis (castor plant) is of great medical importance. Different parts of the plant have unique and distinctive uses. Its leaves are used in the treatment of jaundice, burns, skin infections, pain in joints, hepatoprotective agent etc. The phytoconstituents present in leaves are terpenes, flavonoids, alkaloids, phenols, and saponins etc. The leaves are coppery red in color due to presence of anthocyanins. The ethanolic extract of the leaves obtained using Soxhlet apparatus found to be effective in the treatment of hyperbilirubinemia, which is yellow discoloration of body tissue due to redundant bilirubin in the body. The leaves contain ricinine, fumaric acid, dimethyl ricinine, quercetin, and caryophyllene. Ethanol extract of leaves also showed antimicrobial activity. The leaf extract of Ricinus communis is found to have a new perspective to the treatment of different types of jaundice.

KEYWORDS - Ricinus communis, jaundice, soxhlet apparatus, ricinine, dimethylricinine fumaric acid, caryophyllene, quercetin, hyperbilirubinemia, hepatoprotective and antimicrobial activity.

INTRODUCTION - The Castor plant (Ricinus communis) is by far a plant of significant medicinal origin. The whole plant (seeds, leaves and roots) is being used in some way or other. This plant belongs to family Euphorbiaceae and located all over the country, especially in warm and tropical regions. The leaves have a variety of color range from bright green to red/maroon depending on the area of cultivation. The

leaves are coppery red in color due to the presence of anthocyanins. In present day pharmacology, it is used in the treatments of hyperbilirubemia, asthma, inflammation, analgesia, diabetes, oxidant, tumor, nociceptive and also as a strong hepatoprotective agent (Singh and Geetanjali, 2015). The leaves play a vital role in reducing the redundant bilirubin from the body (Singh and Geetanjali, 2015). The phytoconstituents present in the leaves consist of aldehydes, citric acid, alkanes, beta caryophyllene, gallic acid, quercetin, fumaric acid, ricinine. Essential oils present in leaves are camphor, alpha pinene, camphene, alphone, thujone, 1,8 cineole (Singh and Geetanjali, 2015)

Hyperbilirubemia/jaundice is caused due to an increased level of serum bilirubin in the body showing yellow discoloration of skin, sclera, mucous, tongue, urine. Symptoms of jaundice include loss of appetite, dull pain in the area of liver, constipation, extreme frailty, headache, fever and nausea etc. High bilirubin level indicates GI bleeding, diarrhoea, edema, anaemia but can also become lethargic which causes seizures, psychosis, medullary paralysis, or even death. The variants of jaundice include prehepatic jaundice, hepatic jaundice, post hepatic jaundice depending upon the basis of conjugated or unconjugated bilirubin levels. The causes of jaundice in adults and newborn are different. In adults jaundice occurs due to acute/chronic hepatitis, alcoholism, Gilbert's syndrome, medicine induced hepatotoxicity and autoimmune disorders etc. In newborn babies, it may be due to liver malfunction, internal bleeding, sepsis in infant blood, infections, incongruity between foetal and mother's blood. The basic management for jaundice is found to be low fat diet with proper carbohydrate intake and high- water uptake.

Ethanol extract of castor plant leaf at the dose 100 mg/kg of body weight showed significant recovery in ketoconazole persuaded liver damage in mice (Padmapriya et al., 2012). The whole leaves of castor plant delivered a shield against liver necrosis caused due to carbon tetrachloride (Natuet et al., 1977). The grinded powder of dry leaves is used in response to hepatosuppression caused by carbon tetrachloride. The powder was responsible for the reproduction of liver cells and cure liver parenchyma (Pingale et al., 2010). It may be due to the production of antimicrobial compounds by *Ricinus communis* specifically against gram negative bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa* (AlKuraishy et al., 2012). This study gave the scientific validation to the bioactive compounds present in castor leaves that exhibit highly encouraging hepatoprotective activity.

In the present study, we have evaluated the antimicrobial activity of ethanol extract of *Ricinus communis* planted in the botanical garden of Shreeyash institute of pharmaceutical education and research. The examination was performed on the basis of agar diffusion and minimum inhibitory concentration method. The syrup is prepared for further use in pharmaceutical formulations.

MATERIALS AND METHODS -

Collection of plant leaf sample:

Fresh leaves of *Ricinus communis* were collected from the botanical garden of Shreeyash institute of pharmaceutical education and research, Aurangabad, Maharashtra.

METHOD 1:-**Preparation of extract:**

The fresh leaves of *Ricinus communis* were collected and air dried till the moisture content reached $10 \pm 2\%$. The leaves were kept on a dry cloth and grinded to obtain coarse powder. The extraction process was carried out by utilizing ethanol as a solvent in Soxhlet apparatus. The sample was placed in a disposable thimble and performing the Soxhlet extraction up to 4 cycles. The obtained extract was used for the evaluation of antimicrobial activity of *Ricinus communis*. Inhibition of microbial growth was observed using *Bacillus subtilis*, *Bacillus licheniformis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* on the agar plate.

**Phytoconstituents present in the leaf of *ricinius communis***

phytoconstituents	Functions
Ricinine	hepatoprotective
N-Demethyl ricinine	hepatoprotective
Citronellol	anti bacterial, antispasmodic, hepatoprotective
1,8-cineole	anti inflammatory, antihepatotoxic
Linalol	antimicrobial, analgesic, reduces hepatic damage
α -pinene	hepatoprotective, anti tumor, anti euphoric
β -pinene	bronchodilator, anti inflammatory, anti hepatotoxic
Thymol	ameliorates liver injuries, antibacterial

Primary screening of phytoconstituents-

Phytochemicals are an important aspect of parts of plants derived from a pharmacognostic origin; medicinal plants often are categorized with their phytoconstituents which has distinctive properties. Each phytoconstituent has a different function and different structure chemically. So to distinguish between the

variety of phytoconstituents present in the leaf of *ricinius communis* screening is done with the help of different reagents and tests for various phytochemicals. Phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds. The two main components of the leaf which were involved in the hepatoprotective activity, ameliorating liver damage are ricinine and N dimethylricinine which are alkaloidal in nature. The other compounds found in the leaves of extract were glycosides, carbohydrates, saponins, tannins, flavonoids, triterpenoids etc.

CARBOHYDRATES

TEST	PROCEDURE	OBSERVATION	RESULT
Molish test	The test solution is combined with a small amount of Molisch's reagent (α -naphthol dissolved in ethanol) in a test tube. After mixing, a small amount of concentrated sulfuric acid is slowly added down the sides of the sloping test-tube, without mixing, to form a layer	Purple ring at the junction of two liquids	Carbohydrate present
Benedicts test	Approximately 1 ml of sample is placed into a clean test tube. 2 ml (10 drops) of Benedict's reagent (CuSO ₄) is placed in the test tube. The solution is then heated in a boiling water bath for 3-5 minutes.	No colour change	Non reducing carbohydrate
Barfoeds test	1 drops of Barfoed's reagent is added to 2 mL of given sample in a test tube and boiled for 3 minutes and then allowed to cool	Cherry red colour	Carbohydrates present
Seliwanoffs test	Take two clean, dry test tubes and add 1 ml of the test sample in one test tube and 1 ml of distilled water in another as blank. Add 2 ml of Seliwanoffs' reagent to both the test tubes. Keep both the test tubes in a water bath for 1 min.	Cherry red colour	Carbohydrates present

Tollens test	Take the given sample solution in a clean test tube. Add 2-3ml of tollens reagent to it. Keep the test tube in a boiling water bath for 10 minutes	Silver mirror formed	Carbohydrates present
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GLYCOSIDE-

TEST	PROCEDURE	OBSERVATION	RESULTS
Raymond test	to the drug, add a few ml of 50% ethanol and 0.1 ml of 1 % solution of m-dinitrobenzene in ethanol. To this solution, add 2-3 drops of 20% sodium hydroxide solution.	Violet colour appears	Glycosides present
Legal test	To the drug, add few ml of pyridine and 2drops of nitroprusside and a drop of 20% sodium hydroxide solution	Deep red colour	Glycosides present
Killer killiani test	Glycoside is dissolved in a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid. Add one or two drop of concentrated sulphuric acid	Blue colour	Glycosides present
Xanthyrol test:	The crude is heated with 0.1 to 5% solution of Xanthyrol in glacial acetic acid containing 1% hydrochloric acid	Red colour	Glycosides present
Baljet test:	Take a piece of lamina or thick section of the leaf and add sodium picrate reagent.	Yellow-orange colour	Glycosides present

ALKALOIDS-

TEST	PROCEDURE	OBSERVATION	RESULT
Dragendroff test	Leaf extract solution+solution of potassium bismuth iodide	Orange red precipitate	Alkaloids present
Mayers test	Leaf extract +solution of potassium mercuric iodide	Cream coloured precipitate	Alkaloids present
Wagners test	Leaf extract +solution of iodine in potassium iodide	Reddish brown precipitate	Alkaloids present
Hagers test	Picric acid +leaf extract	Yellow precipitate	Alkaloids present

PHENOLS

TEST	PROCEDURE	OBSERVATION	RESULT
Ferric chloride test	Leaf extract + ferric chloride solution	Voilet colour formed	Phenols present
Libermans test	Leaf extract+conc sulphuric acid+sodium nitrite	Deep blue colour	Phenols present
Bromine water test	Leaf extract+bromine water	Brown colour	Phenols present
Phthalein dye test	Leaf extract+phthalic anhydride Mixture treated with sodium hydroxide	Pink colour formed	Phenols present

STEROIDS

TEST	PROCEDURE	OBSERVATION	RESULT
Salkowski test	2mg of dry extract shaken with chloroform, sulphuric acid slowly added by the sides	Formation of red colour	Steroids present
Lieberman-buchards test	2mg of dry extract dissolved in acetic anhydride heated and then cooled and 1ml of sulphuric acid concentrated was added	Formation of green colour	Steroids present

SAPONINS-

TEST	PROCEDURE	OBSERVATION	RESULT
Saponin test (1)	5ml leaf extract+ 5ml of water + heat	Froth appears	Saponins present
Saponin test (2)	5ml leaf extract+ few drops of olive oil	Emulsion forms	Saponins present

Tannins-

TEST	PROCEDURE	OBSERVATION	RESULTS
Leaf sample + $FeCl_3$	Greenish colour formed	Catechol suspected	Tannins may be present
Leaf sample + acetic acid+ lead acetate solution	Slight precipitate formed	Pyrogallol/gallic acid suspected	Tannins may be present

Leaf sample+ conc H ₂ SO ₄	Formation of red coloured ring at the junction	Catechol may be present	Tannins may be present
Leaf sample+ acetic acid + bromine water	Formation of precipitate	Catechol confirmed	Tannins confirmed

FLAVONOIDS-

TEST	PROCEDURE	OBSERVATION	RESULT
Alkaline reagent test	Leaf extract + few drops of sodium hydroxide solution	Formation of intense yellow colour	Flavonoids present
Lead acetate test	Leaf extract + few drops of lead acetate solution	Yellow precipitate	Flavonoids present
Ferric chloride test	Leaf extract + FeCl ₃ solution	Intense green colour	Flavonoids present

TRITERPENOIDS-

TEST	PROCEDURE	OBSERVATION	RESULT
Libermann – buchards test	Leaf extract+ acetic anhydride boil and cool+ conc sulphuric acid from the side of the test tube.	Brown ring forms at the junction of two liquids the lower layer is deep red in colour	Triterpenoids present
Salkowski test	Leaf extract +conc H ₂ SO ₄	Yellow colour	Triterpenoids present
Sulphur powder test	Leaf extract solution + A small amount of sulphur powder	Sulphur powder sits at the bottom of the test tube	Triterpenoids present

Isolation of bacteria:

A soil sample was collected from the Shreeyash college garden. One gram of soil was mixed with 100 ml of water (stark solution). A serial dilution method was used to reduce a dense culture of cells to a more usable concentration. In this method, 5 test tubes were taken containing 9 ml of water, 1 ml of stark solution was added in a test tube to achieve 1/10th dilution. Successive dilutions were performed for 5 times. The diluted samples were subjected to agar plate.

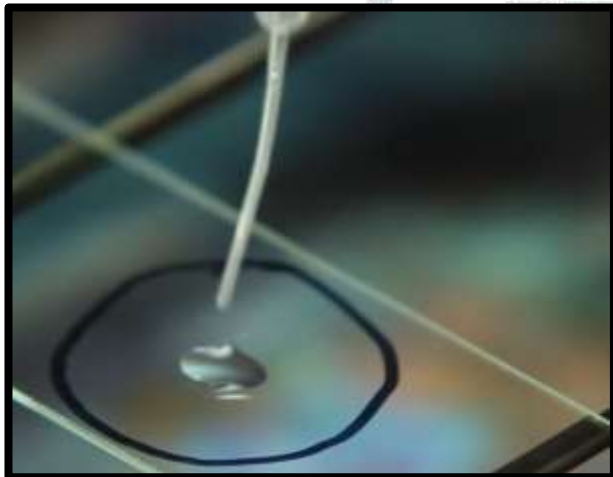
Preparation of agar plates

Agar plates were prepared using a media containing agar powder (20 g/L), peptone extract (5 g/L), beef extract (3 g/L), and yeast extract (3 g/L) in distilled water. All the components were mixed and autoclaved at 121°C for 15 min. Plates were prepared using 10 ml of autoclaved media in each plate. These agar plates were inoculated with serially diluted samples (1 ml) and incubated at 37 °C for 48 h – 72h to develop the colonies. The same method of serial dilution was performed for the wastewater (sewage) by taking 1 ml of sample to 100 ml pure water.



Identification of bacteria:

The best method for staining of bacteria is Gram staining, which gives an idea about whether the bacteria is Gram positive or Gram negative. A bacterial smear was prepared on a slide and air dried for few min. The slide was heated onto a burner to adhere smear firmly to take the stain. Crystal violet was poured generously on the heat fixed slide for about 2 min, and then added Gram's iodine. The slide then was treated with ethanol for about 40 s and further flooded with safranin for 2–3 min. The slide was washed with distilled water and observed under an oil immersion microscope. Gram staining is used to identify the gram nature of the bacteria(gram positive or gram negative). Since only this characteristic of bacteria isn't enough a wide range of other characteristics is also evaluated based on some prominent parameters ,these parameters help analyse where exactly does the bacterial species belong and where they can be grouped together. The isolated colonies can be studied via its **colour** (white, pale yellow, bluish green , pinkish yellow etc), **shape** (rod shaped, coned shaped, spiral shaped,circular shaped etc),**enclave** which refers to the morphology of the colonies (opaque disc , white hair like ,smooth round etc) , consistency (dry,cloudy,mucus) , **margin** (irregular or entire) , **motility** (sliding motility, non motile, motile etc).





Method2 :

Preparation of syrup: Fifty grams of the coarse powder of *Ricinus communis* was added in 400 ml of water. This mixture was reduced down until the water becomes thick in nature and was measured up to 100 ml. This form of extraction is called as decoction where in a concentrated form of liquor is formed which is the result of boiling or heating a medicinal preparation made from a plant (the extract can be made up of leaves, roots, barks of plant etc.). The extract was used to in sucrose and honey syrup preparation. The decoction now had medicinal properties and can be used for making pharmaceutical formulations.

- **Sucrose syrup preparation:** Sucrose (33.33 g) was accurately weighed and fused in 100 ml of water initially with slow heat on the Bunsen burner for few min, then further the amount of heat was increased until formation of a viscous preparation of syrup. The extract was added in the syrup with the ratio of 1:5 .Few drops of lemon juice were also added as a rich source of vitamin C, which is a strong immunity booster (Wintergerst et al., 2006). Sodium benzoate (0.2%) was added as a preservative and the extract was kept in a cool and dry place away from sunlight.



- Honey syrup preparation: Honey (50 g) was added to 100 ml of distilled water while increasing temperature slowly till preparation becomes consistent syrupy. The extract and the honey preparation were mixed in the ratio of 1:5 with few drops of lemon juice and also 0.2% sodium benzoate. The extract was transferred to a clean container and stored away from sunlight in a cool and dry place.



Calculations:- The experimental calculations for density, viscosity and ph of sucrose and honey preparation were done using the Ostwald viscometer, density bottle method and ph meter respectively.

Sucrose syrup (viscosity):-

Liquid parameters	Flow time in seconds			Total time
	1	2	3	Mean score
Distilled water	410	400	420	410
sucrose syrup	102	103	105	103

Weight of the empty bottle = 25.75 (W1)

Weight of bottle + distilled water = 54.60 (W2)

Weight of bottle + sample = 56.66 (W3)

∴ Weight of the liquid = $W3 - W1 = 56.66 - 25.75 = 30.91$

Weight of distilled water = $W2 - W1 = 54.60 - 25.75 = 28.85$

$$\begin{aligned} \therefore \text{density of the given liquid} &= \frac{\text{weight of liquid}}{\text{weight of distilled water}} \times \text{density of water} \\ &= \frac{30.91}{28.85} \times 0.9998 \end{aligned}$$

Density of sucrose sample = 1.692

Now, to determine the viscosity of given liquid, $= t_1 p_1 / t_2 p_2 \times \eta_w$

t_1 = mean time of sample(sucrose)

t_2 = mean time of distilled water.

p_1 = density of sample(sucrose).

p_2 = density of distilled water.

η_w = viscosity of water. = 0.894

$$\eta = t_1 p_1 / t_2 p_2 \times \eta_w$$

$$\eta = \frac{103 \times 1.07}{410 \times 0.998} \times 0.894$$

\therefore viscosity of sucrose sample was 0.2398



viscosity measured with Ostwald viscometer



The ph of given sucrose syrup was 7.19

Honey syrup (viscosity determination):-

Liquid parameters	Flow time in seconds			Total time
	1	2	3	Mean score
Distilled water	409	400	419	409
Honey syrup	72	73	72	72

Density calculation (honey)-

To determine the density of given liquid ,

Weight of the empty bottle = 26.80 (W1)

Weight of bottle + distilled water = 53.51 (W2)

Weight of bottle + sample = 55.49 (W3)

∴ Weight of the liquid= $W3 - W1 = 55.49 - 26.80 = \underline{28.69}$

Weight of distilled water = $W2 - W1 = 53.51 - 26.80 = \underline{26.71}$

$$\begin{aligned} \therefore \text{density of the given liquid} &= \frac{\text{weight of honey}}{\text{weight of distilled water}} \times \text{density of water} \\ &= \frac{28.69}{26.71} \times 0.998 \end{aligned}$$

Density of honey sample= 1.0739

Now, to determine the viscosity of given liquid, $= t_1 p_1 / t_2 p_2 \times \eta_w$

t_1 = mean time of sample (honey).

t_2 = mean time of distilled water.

ρ_1 = density of sample.(honey).

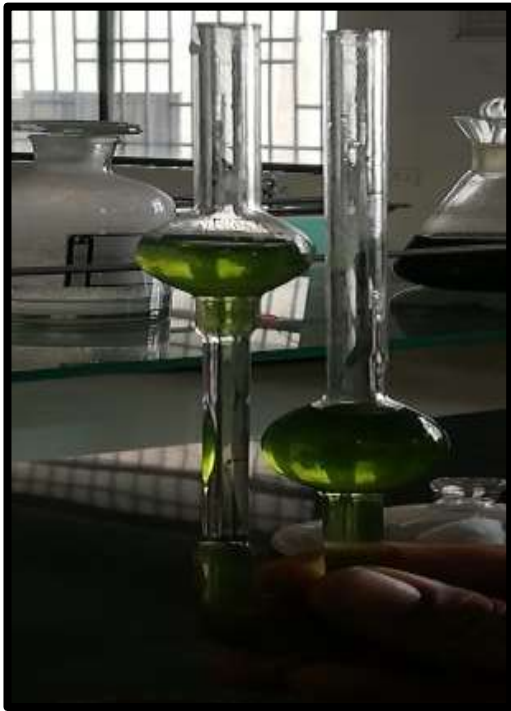
ρ_2 = density of distilled water.

η_w = viscosity of water. = 0.894

$$\eta = t_1 \rho_1 / t_2 \rho_2 \times \eta_w$$

$$\eta = \frac{1.07 \times 72}{409 \times 0.998} \times 0.894$$

\therefore viscosity of honey sample = 0.1680



ph of given sample of honey is 7.51



Results and discussions-

Ethanol extract of *ricinus communis* which was used for evaluating the antimicrobial activity was found to inhibit *staphylococcus aureus* , *Eischeria coli* , *Bacilius subtilis*, *Bacillus licheniformis* , *Pseudomonas aeruginosa*.

The two types of extract were prepared because ethanol can be used as an excellent solvent but is hazardous to the human health so it could not be administered to the body. The ethanol as well as the water extract showed same phytoconstituents and primary screening also after evaluation had same results.

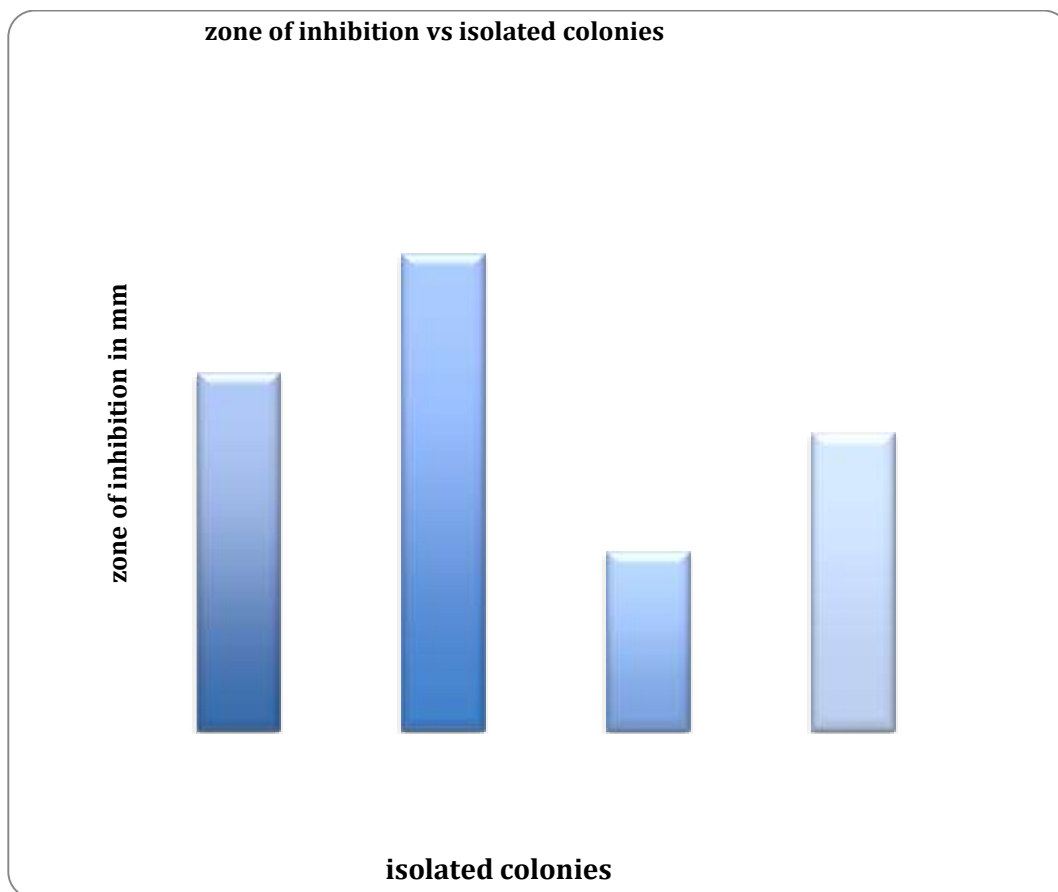
The syrup of *ricinius communis* prepared using sucrose and honey (natural sweetner for diabetic patients) had various pytoconstituents present in the leafs.

Zone of Inhibition:- (Table no.1)

Isolated colonies	Zone of inhibition in diameter (mm)
B1 Bacillus subtilis	0.6mm
B4 Bacillus licheniformis	0.8mm
Staphylococcus aureus	0.3mm
Eischerichia coli	0.5mm
Pseudomonas aeruginosa	no growth

zone of inhibition –this is an area of media where the bacteria is unable to grow due to presence of some inhibiting factors such as drugs more precisely antimicrobial agents

Isolated colonies – when lone bacterial cells divide and give rise to thousand of new bacterial cells it is called as the isolated colony of bacteria.

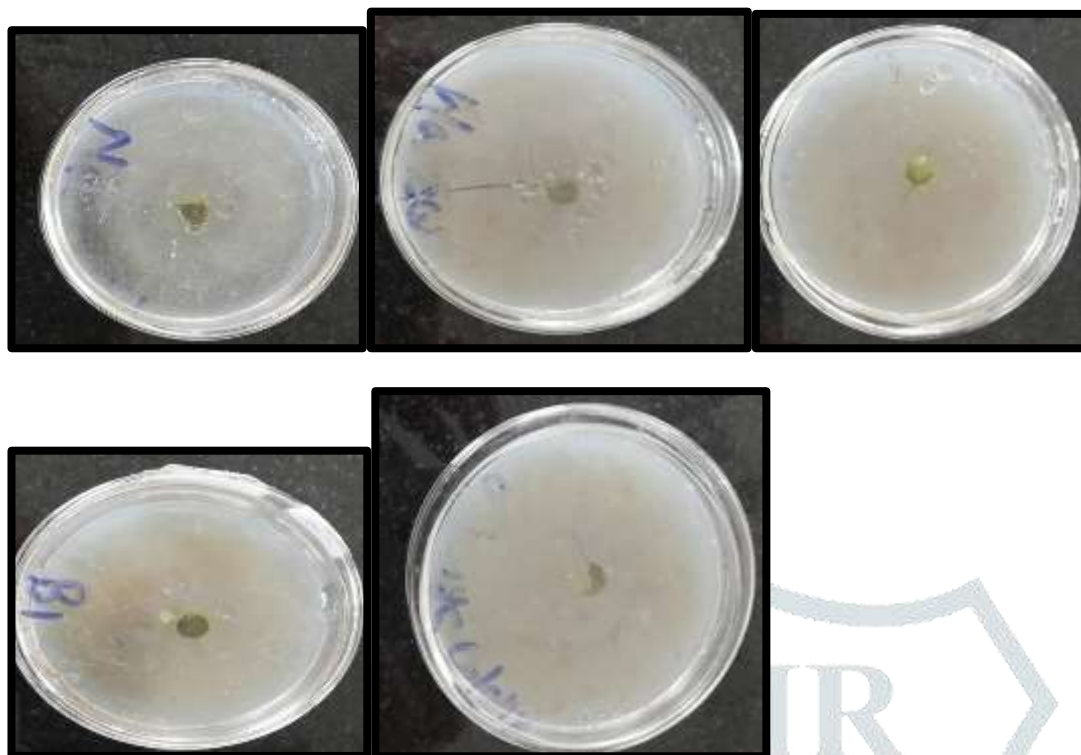


Isolated colony characteristics:- (table no.2)-

These are used for distinctive characterization of bacterial species it helps to differentiate between different species through which identification becomes much easier and simpler.

parameters	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
Colony	B1 bacillus subtilis	B4 bacillus licheniformis	staphylococcus aureus	Escherichia coli	Pseudomonas aeruginosa
Colour	Fuzzy white	Pale	Golden yellow colour	Off white	Greenish colour
Shape	Rod shaped	Thin rod shaped	Spherical shaped	Rod shaped	Rod shaped
Enclave	Rough opaque disc	White hair like	Grape like colonies	White translucent disc	Smooth round
Consistency	Dry	Dry	Buttery	Mucus/ cloudy	Mucoid
Margin	irregular	irregular	entire	entire	Irregular
Gram nature	Gram positive	Gram positive	Gram positive	Gram negative	Gram Negative
Motility	Sliding motility	Motility	Non-motile	Motile	Motile





Comparison of standard preparation with honey and sucrose –(Table no 3)

parameters	standard syrup	preparation 1 (sucrose)	preparation 2 (honey)
Appearance	clear	clear fluid like	turbid syrupy thick
Colour	yellowish green	orange yellow	dark green
pH	6.15	7.19	7.51
Taste	sweet	sugary sweet	honeyed sweet
Viscosity	3.06	0.237	0.1680
Density	1.06	1.692	1.0739
Specific gravity	1.17	1.040	1.114

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