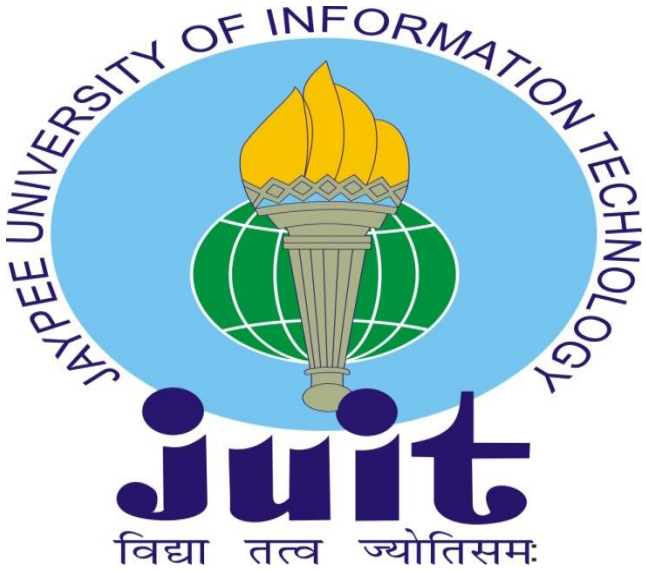
To determine the potential of *Strychnos potatorum* for management of portable water

PROJECT REPORT BY:

Lakshay Sharma (161803)

UNDER THE SUPERVISION OF

DR. UDAYBANU



DEPARTMENT OF BIOTECHNOLOGY AND BIOINFORMATICS

JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY

WAKNAGHAT, SOLAN- 173234, HIMACHAL PRADESH

CERTIFICATE

I, hereby declare that the work presented here in this report entitled "**To determine the potential of *Strychnos potatorum* for management of portable water**" in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the department of Biotechnology and Bioinformatics, Jaypee University of Information Technology Waknaghat is an authentic record of my own work carried out over a period from August 2019 to December 2019 under the supervision of Dr. Uday Banu. The matter embodied in the report has not been submitted for the award of any other degree or diploma.

Lakshay Sharma (161803)

This is to certify that the above statementmade by the candidate is true to the best of my knowledge.

Dr. Uday Banu

Dated:December 2nd,2019.

ACKNOWLEDGEMENT

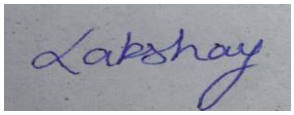
We are thankful and grateful to our guide Dr. Udaybanu and our PhD Scholar Diksha Manhas for their extraordinary and brilliant guidance, constant and valuable feedback along with the continuous encouragement throughout the duration of our major final year project. We are thankful to him for providing us this wonderful opportunity to work in his esteemed lab.

We are highly thankful to Diksha for her constant support during our project work and for her continuous guidance, mentorship.

We are also thankful to Dr. Sudhir Syal for his valuable assistance and guidance and providing liberty to do good and quality experimental work.

DECLARATION

I, hereby declare that the project work entitled "**To determine the potential of *Strychnos potatorum* for management of potable water"** submitted to the Department of Biotechnology(BT) and Bioinformatics(BI), Jaypee University Of Information Technology Solan(H.P), is an authentic record of original work done by us . The work was carried out the under the guidance of **Dr. Udayabanu .** This work has not been submitted anywhere else.



Signature

LAKSHAY SHARMA (161803)

Date:

This is to certify that the above statement made by the candidates is correct to the best of my knowledge.

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Dr. Udayabanu

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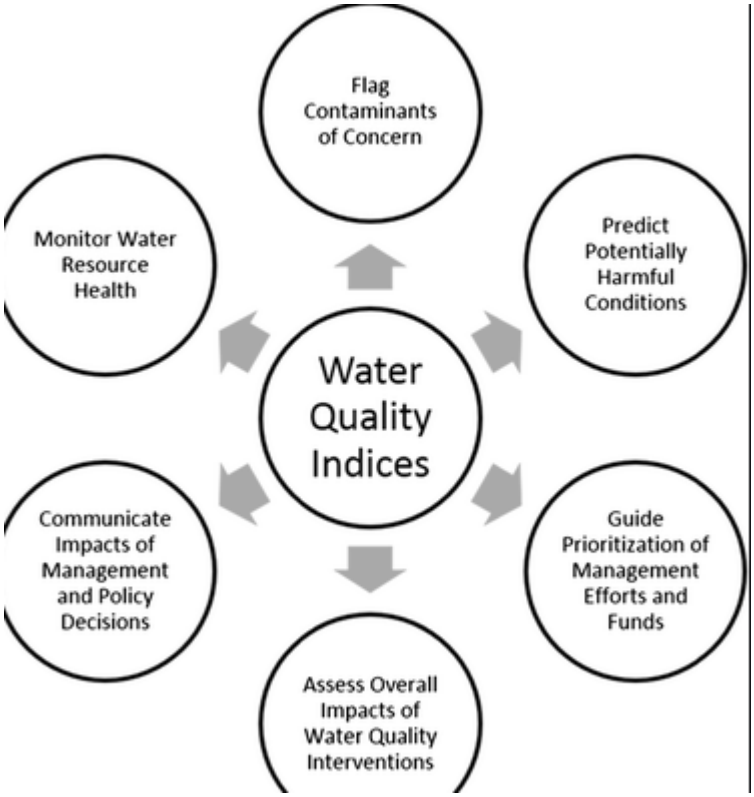
ABSTRACT-

The plan to treat water by regular coagulants is numerous hundred years old. Common coagulant like *Moringa olifera, Mangifera indica, Strychonas potatorum* are utilized for the treatment of waste water. Nirmali seeds contain normal regular coagulant with polyelectrolytes. These are liable for coagulation properties. Our examination planed for discovering efficiency of nirmali seeds as regular water treatment as a choice to utilization of synthetic compounds for water treatment. The viability of nirmali seeds in the evacuation of turbidity, pH, TDS was to be explored. Primer phytochemical screening was completed. Movement of nirmali seeds was to be analyzed against different coliforms microscopic organisms found in water test. The essential objective of this examination was to discover the attainability of utilizations of nirmali seeds for rewarding tainted water tests.

CHAPTER 1 –INTRODUCTION

1.1 Introduction

Water purity is very essential as water is basic foundation of life and effects human health directly or indirectly. Traditionally water was treated through sedimentation, filtration and disinfection. Treating water is a major concern all over the globe in every country. Every human being depends on water for drinking and other needs. Development of large infrastructure has contaminated natural water resources on a large scale. Many human activities like washing cloth, bathing on river sides have polluted the water bodies to a large extent. Six million people die because of diarrhoea every year. Almost 23000 people die of drinking contaminated water every year in developing countries. Chemicals used to disinfect water produces various undesirable disinfection by products or DBPs. Due to these problems there is urgent need to find new water purifying techniques at low cost and with lower energy, which will reduce the impact of chemicals on environment. Since many years water has been traditionally treated naturally through herbs in India. Seeds of Elettaria cardamomum, ripe seeds of Strychnos potatorum, seeds of Moringa oleifera were used in water treatment. We are highlighting the use of nirmali seeds in water treatment. S.potatorum belongs to the family loganiaceae. The plant is mainly cultivated in asia and Africa. In Mayanmar, Sri Lanka, India it is also used as medicinal extract. It is generally a deciduous tree upto a height of 12 metere. Bark is thick, leaves are elliptic, and white fragnant flowers. Useful parts of the plant are seeds, roots and fruits. The plant has many ayurvedic properties including treatment of chronic diarrhoea. It also cures belching problem, useful for people suffering from various attacks of cough. Its seed in combination with honey and camphor cures lachrymation. Ripe fruits and seeds are used as antidotes against snake bite. Seeds can also cure strangury, urinary discharges and head diseases. Seeds are generally alexipharmic, acrid and lithotriptic. Its roots are believed to treat leukoderma and fruits used to treat eye diseases, poisoning, thirst, halucinations. According to Unani system of medicine it is good for kidney and liver complaints, gonorrhoea. Fatty oil from seeds is used in paints, soaps and detergent manufacturation. Ripe seeds settle the colloidal matter present in water . Trees can withstand severe drought and act as **shelter belts against strong winds and purifies air too.** Seeds are used to extract polysaccharide gum which is used in paper and textile industries. Seeds are also used to treat urolithiais, polyuria, and dysuria.



1.2 TAXONOMIC CLASSIFICATION

- **Kingdom-Plantae**
- **Class-Angiosperms**
- **Subclass-Eudicots**
- **Superorder-Asterids**
- **Order-Gentianales**
- **Family-Loganiaceae**
- **Genus-Strychnos**

1.3 COMMON PLANT NAMES[1,2]

English- clearing nut tree

Sanskrit- Katakarenu, payaprasadi, Rushya, Tiktaphala, Lekhanatmaka, Tiktamaricha, Chakshushya, Guchhaphala, Kattha, Khataka.

Hindi- Nirmali, Neimal, Nelmal.

Bengal- Nirmali

Marathi- Gajara, Nirwali, Chilhara.

Punjabi- Niemali

Tamil- Sillam, Tatta, Tettankottai, Ilalam

Malayalam- Tetta, Tettamparap, Katakam.

CHAPTER 2 -REVIEW OF LITERATURE:

Water is need for our every day life processes. Yet, access to clean drinking water isn't accessible to a significant populace living in country zones. In India, significant populace drink water from unprotected sources. These days in excess of 1100 million individuals live deprived of access safe drinking water. As indicated in report by WHO, there are roughly 2 million diarrhoeal passings identified with hazardous water. People are prime driver of water contaminations, which activated from plentiful points of view: by the dumping of mechanical waste, because of temperature risees, that causes the adjustment of water by decreasing the oxygen in it piece, or because of deforestation, which makes silt and microscopic organisms show up under the dirt and consequently sully ground water. Similarly, the pesticides utilized in agrarian fields channel through underground channels and arrive at the utilization arrange. The common happening component present at unsuitable levels can pollute water too. Different contaminants are man made side-effects of industry, and horticulture including overwhelming metals like mercury, copper, chromium, lead and unsafe synthetic concoctions, colors and mixes like bug spray and manures. As indicated by report by UN consistently 2 million tons of sewage, mechanical and agribusiness squander are released into the universes water. The microbial contaminants incorporate microorganisms like microorganisms, infections and parasites, for example, minuscule protozoa and worms. These living life forms can be spread by human and creature squanders purposely or unwittingly. Essentially the water contaminants related with water sullying are of four sorts:

- Inorganic contaminants (fluoride, arsenic, lead, copper, mercury)
- Organic contaminants (pesticides, domestic wastes, industrial wastes)
- Biological contaminants(algae, bacteria, protozoans, viruses)
- Radiological contaminants(uranium, radium)

Traditional ways of water purification includes boiling, filtration, sedimentation, long storage and solar radiationDespite the fact that these customary strategies are convenient and can

evacuate particular kinds of molecule in water, they don't give water essentially of what might be considered under the current day circumstance as drinking quality, that is satisfactory to country networks and in a large portion of the cases, with a further basic advance of cleansing, they could yield water free from pathogens.[4,5]

BOILING

It is customary methods for rewarding water. Whenever done suitably can give harmles water to populace that has no other option. Boiling will slaughter all germ that cause illness. Bubbling annihilates water borne disease causing agents present in water, that is spores, cysts, ova and bacteria and it is equally effective whether the water is cloudy or clear, pure or contaminated with organic matter. It is a satisfactory method for rural areas.

1. ## FILTRATION

   Filtration of water is important initial step , whenever done accurately, will recover viability of the substantal number techniques murmaring slopy grimy loking water through bit of well, unsoiled cotton material utilized to evacuate a specific measure of suspended solids and bug hatchlings contained in water. The fabric ought not be thick to such an extent that it requires some investment to channel the water. It makes family unit water treatment simpler. It relies upon pore size of sifter and stream of water through a strainer on which the insoluble particles are held.

2. ## SEDIMENTATION

   Sedimentation is mainly reduction in turbidity and the efficiency of sedimentation depend on the nature of suspended particle size and allowed setting time. It is apreciated for exclusion of turbidity, pathogenic microbes.

3. ## SOLAR DISINFECTION

Presenting water to daylight will devastate most germs that causes maladies. This is much increasingly viable at higher temperature. In tropical areas, a protected presentation period is

around 5 hours, based on noontime. The measure of time the container presented to sun should be multiplied when the water is cloudy.It takes additional time than different techniques and requiresunny climate.

DRAWBACKS OF TRADITIONAL WATER PURIFICATION METHODS

Although effective in ridding water of some substances, these methods still leave behind harmful materials in the water. Also pregnant women or those with thyroid condition should not drink water with chemicals. Chlorine is very poisonous and adding too much can cause illness. Methods like boiling consume lots of fuel and there is loss of water in evaporation.

PLANTS A NEW APPROACH TOWARDS WATER PURIFICATION

Tending to the issues talked about above there is a requirement for huge measure of examination to be directed to distinguish powerful new strategies for purging water at low cost and les vitality, simultaneously limiting utilization of synthetic substances sway on the earth. santimicrobial exercises, coagulation and different exercises. Characteristic polyelectrolytes of plant root have been utilized for a long time. For consederable length of time surface water dealt with generally utilizing spices as normal coagulants in India. Ready seeds of strychnos potatorum, from phyllantus emblica were well known for their utilization in water treatment. Another regular option for water refinement is the seeds of Moringa oleifera .There use has been utilized for a long time as an ease supplement enhancer in the under favoured nations around globe.

Strychnos Potatorum

Strychonus Potatorum otherwise called clearance nut shrub spread all through tropical just as sub tropical district of world .Strychonus potatorum seeds are broadly utilized in ayurvedicand conventional prescriptions .Apart from its clinical properties it is likewise being utilized for clearing sloppy water from rustic network .They accounted to extremely viable characteristic coagulants helps . This possession ascribed in view of nearness of polyelectrolyte ,protiens

,lipids ,starches and alkaloids containing the COOH and OH surface gatherings in the seed .this property of seed is utilized to treat the different kind of savoring water request to recognize its potential against antimicrobial movement .

The ratings of water quality can be defined bby following table

National Sanitation Foundation Water Quality Index (NSFWQI)	
WQI Value	**Rating of Water Quality**
91-100	Excellent water quality
71-90	Good water quality
51-70	Medium water quality
26-50	Bad water quality
0-25	Very bad water quality
Canadian Council of Ministers of the Environment Water Quality Index (CCME WQI)	
95-100	Excellent water quality
80-94	Good water quality
60-79	Fair water quality
45-59	Marginal water quality
0-44	Poor water quality
Oregon Water Quality Index (OWQI)	
90-100	Excellent water quality
85-89	Good water quality
80-84	Fair water quality
60-79	Poor water quality
0-59	Very poor water quality

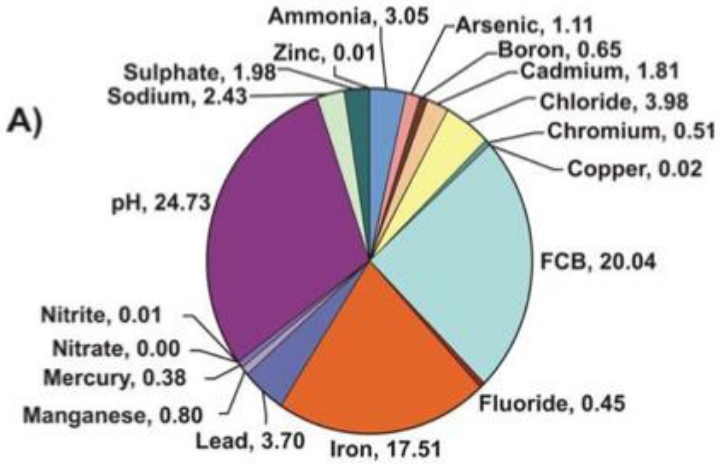
Now ratings for water quality as per Weight Arithmetic water quality Index method

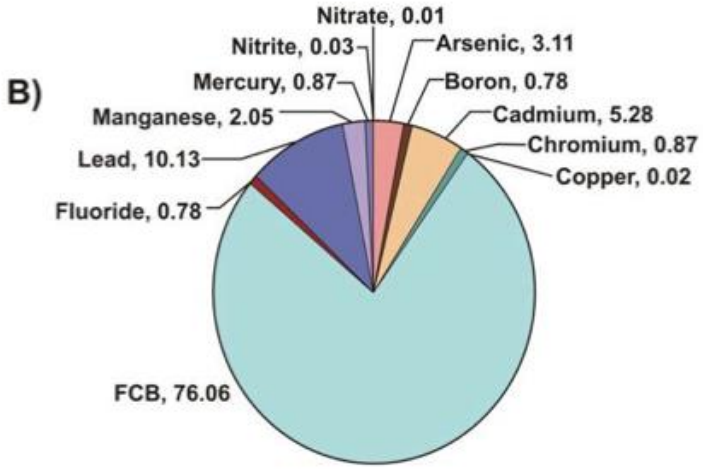
WQI Value	Rating of Water Quality	Grading
0-25	Excellent water quality	A
26-50	Good water quality	B
51-75	Poor water quality	C
76-100	Very Poor water quality	D
Above 100	Unsuitable for drinking purpose	E

According to CCME

CCME WQI	Classification	Description
95-100	Excellent	Water quality is protected with virtual absence of threat or damage. The conditions are very close to natural levels or desired.
80-94	Good	Water quality is protected with some threats or damage of low magnitude. Conditions rarely depart from the desired or natural levels.
65-79	Regular	The water quality is usually protected but occasionally threatened or damaged. Conditions sometimes depart from the desired or natural levels.
45-64	Marginal	Water quality is frequently threatened or damaged. Conditions frequently depart from the desired or natural levels.
0-44	Poor	Water quality is almost always threatened or damaged. Conditions usually depart from natural levels or desired

Global drinking Water Quality Index





Principle and Procedure of Certain Water Parameters

Acidity -

Principal -

- Acidity basically checks the presence of hydrogen iions on hydrolysis of solute when it reacts with standard alkali .
- It depends on the end point of indicator used .
- The colour change of Phenophthalien corresponds to stoichiometric neutralization of Carbonic acid to bicarbonate .

CHAPTER 3 - MATERIAL AND METHODS

3.1 Plant Sample Collection

Plant sample nirmali seeds was gathered from district Ramnad which is present in Tamil Nadu.

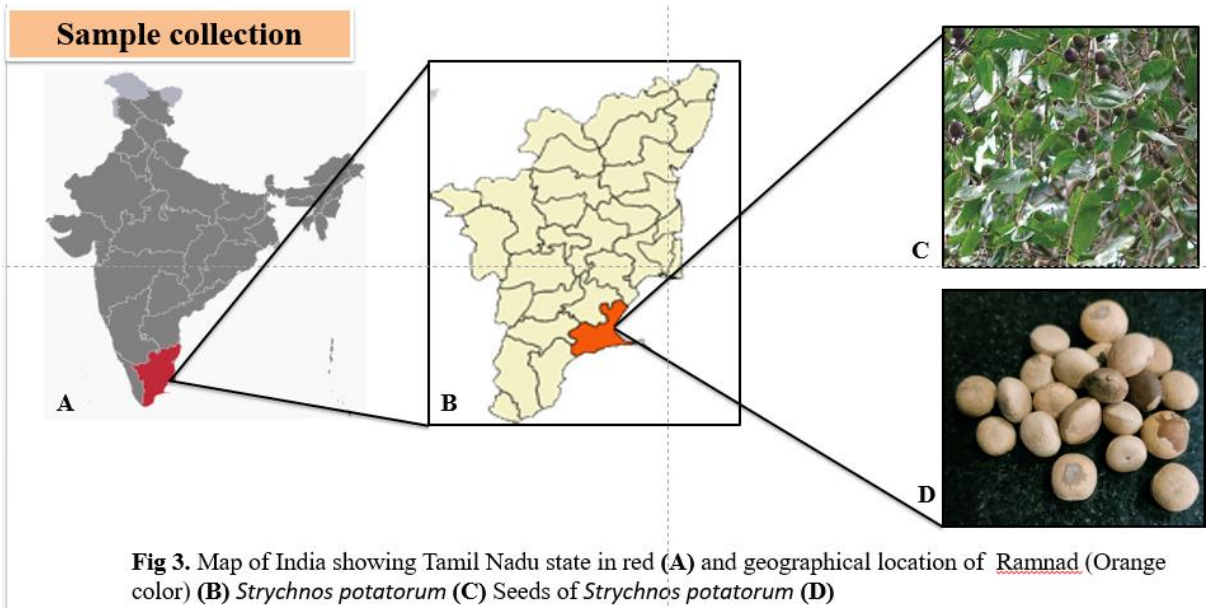


Fig 3. Map of India showing Tamil Nadu state in red **(A)** and geographical location of Ramnad (Orange color) **(B)** *Strychnos potatorum* **(C)** Seeds of *Strychnos potatorum* **(D)**

3.2 Preparation of Solvent Extract of *S.potatorum*

The plant extract were prepared manually .The following solvents were used for extract preparation. Aqueous and ethanol . 2 gm of dried plant seeds was mixed with respective solvent 1:10 ratio and reared for 24 hours at area temperature .extract was vortexd for 10 minutes. Centrifuged at 14000 rpm for 10 minutes and supernatant collected in fresh tube .the extraction with the residual plant powder was repeated 2 times with the same solvent. The supernatant were pooled and extract was dried at 40 degree and evaporated to dryness and stored at 4 degree.

3.3 Phytochemical Screening

Alkaloids -

1 ml plant extract mixed with limited drop of mayer's reagent test tube .The appearance yellow precipitate indicated presence of alkaloids .

Flavanoids -

1ml plant extract mixed with 1 ml of NaoH . Then some drops of dilute HCLwere added . change in color of solution from yellow to colourless displayed atendance of flavonoids in plant extract .

Saponin -

1ml of extract ,1 ml of distilled water in test tube was added and shaken vigorously .Froath formation indicated the presence of saponins .

Tannins -

1ml plant extract mixed with few drops of 10% lead acetate .Precipitate at the bottom of the test tube indicated the presence of tannins .

Phenols -

1ml plant extract was mixed with 3 ml of distilled water .A few drop of Fecl3 was added and appearance of dark green colour indicated the presence of phenols .

Terpenoids -

5ml extract was assorted with 2ml chloroform in a test tube ,then 3 ml of conc. H2SO4 was added dropwise to the edge of test tube . reddish brown rings at interphase of two films would indicate the presence of terpenoids .

3.4 Sampling site

The sample was collected from Ashwani khad near Solan ,Himachal Pradesh .

3.5 Physico Chemical Analysis of Drinking Water

Colected sample was tested for disimilar Physico –Chemical parameter such as pH ,TDS , Strength (Acidity).

Procedure

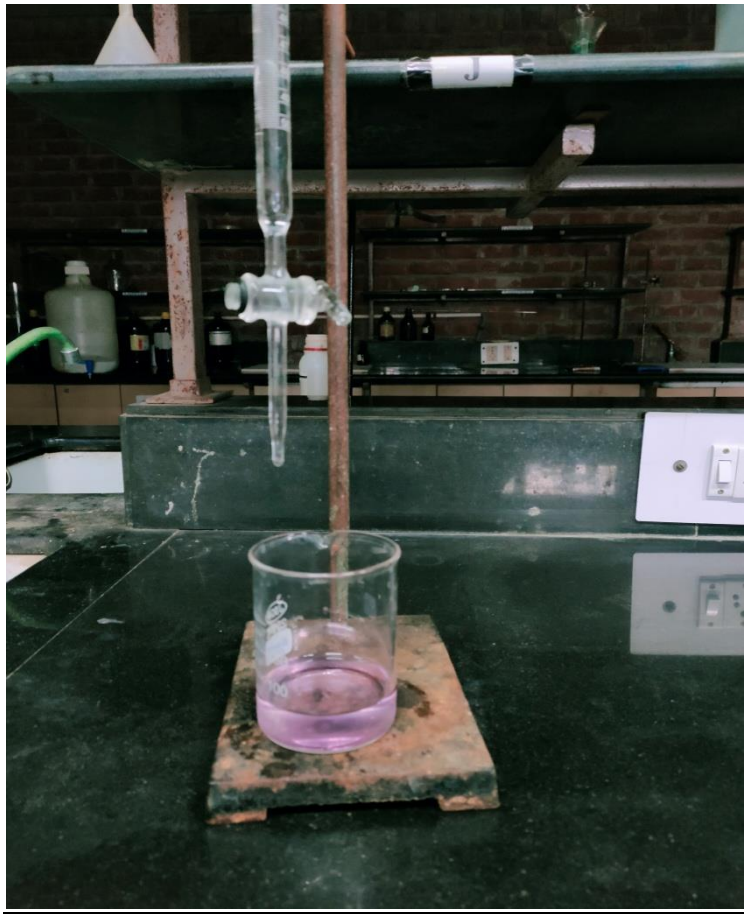
1. Prepartion of Reagants

1.1 Prepartion of 0.02 N NaoH -

1000 ml standard flask is filled with 3/4th distilled water .Sulphuric acid of 1N solution is made using pipette and this is now added to 1000 me standard flask containing distilled water .Total make up1000 ml using distilled water .

1.2 Phenophthalien indicator -

- Weighed 1 gm of phenophthalien and dissolved it in 95% ethyl alchohol



Testing the sample

0.02 sodium hydroxide was put in the burette and burette is adjusted. Fixed the burette. For highly concentrated sample ,usually 100 ml of given sample was taken in flask .Methyl Orange indicator was added in flask . colour changes to orange ,titerated the sample against 0.02 N Naoh till the colour faints .V1 consumed is noted down for calculating mineral acidity .To the same flask add few drops of phenophthalien indicator .Continued titration till colour gets pink .Noted down V2 consumed for next titration. Repeated till you obtain the titration for average value. Due to the presence of indicator the carbonate and bicarbonate ions changed to blue colour and after addition of sulphuric acid colour changed to red indicating that both the ions were neutralized .

Procedure

Preparation of Reagant

1. Sulphuric acid

   Pipetted 20 ml of 0.1 N Sulphuric acid and added gradually along sides of regular flask. The volume was made up to 1000 ml .
2. Phenolphthalein preparation

   1gm of Phenolphthalein was added to 100 ml of 95% ethyl alcohol.

Procedure

Fixed the burette with 0.02N sulphuric acid and adjusedt it to 0. Fixed burette in standpoint . 100 ml of sample was dispensed in conical flask. Added few drops Phenolphthalein indicator in the conical flask. Colour changed to pink due to presence of hydroxyl ion. 0.02 N Sulphuric acid was added till the colour disappeared at that particular reading ,noted down V1. A mixed indicator was added in the conical flask and the solution turned blue. Now again titerated till solution becomes red .Noted it as V2. Repeated the titeration to get average .

Caliberation of Ph

Ph electrode is basically combined glass electrode i.e detecting half cell and reference . These collectively form electrode system. Detecting half cell seperates solution into outer solution and inner solution. The difference in potential developed outside and inside is measured as pH of the sample .

Procedure

- Electrode was rinsed in distilled water and then pH of water sample was measured

3.6 Water Testing

1. Preparation of EMB Agar -

A certain amount of agar was disolved in 100ml distilled water. Made certain you blend it well in order to attain a unchanging suspension. Get to boil so that the medium mixed completely. Sterlized the medium by autoclaving 121 degree Celsius for 15 minutes. Made certain you do not overheat the medium. Allowed the medium to cool and shook to oxidize ethylene blue and dangle the precipitate.

3.7 Determination of solids in water and wastewater.(10,11)

AIM-

Determine total solids in given water sample.

INTRODUCTION-

While referring to solids it indicates suspended material dissolved in water that can be isolated physically through filtration or evaporation. Solids are measured as residues left behind upon evaporation of free water.

PRINCIPLE

The sample is allowed to evaporate in a weighed dish on a steam bath and is dried to a constant mass at 103-105 degree C in an oven.

Total solids/residues is measured by calculating increase in mass.

MATERIALS REQUIRED

1. Hot Oven
2. Analytical Balance
3. Desiccators
4. DishTongs
5. Magnetic Stirrer
6. Wash Bottle
7. Crucible

PROCEDURE

Noted down early dry weight of crucible. Took 20ml water sample in crucibles. Placed the crucible inside the oven 103 degree C. Next to drying in oven cool to room temprature in desicator. Write down closing dry weight of crucibles.

CHAPTER 4- RESULTS

Plant sample collection

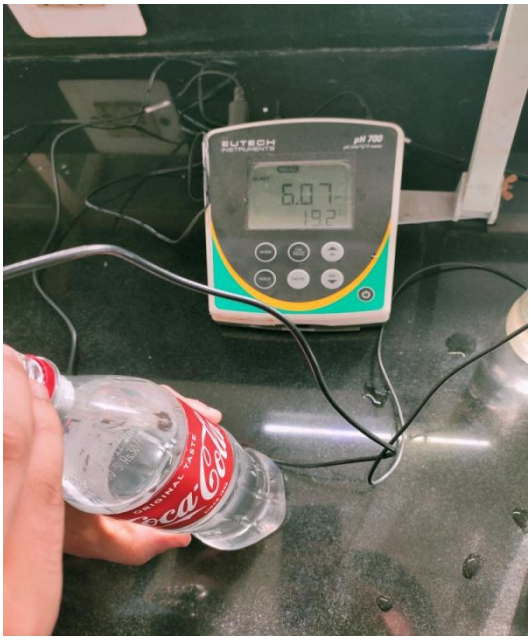
The Plant sample was collected successfully .

Preparation of Plant Extract

1. The preparation of Plant Extract was successfully done in Ethanol and Aqueous solution .

Phytochemical Screening

S.No.	Test	Interfernce	Results
1.	Alkaloids	Yellow Precipitate formed .	Positive
2.	Flavanoids	Yellow to colorless	Positive
3.	Saponins	Froath Formation	Negative
4.	Tannins	Precipitates at the bottom of Test tube .	Positive
5.	Phenols	Appearance of Dark Green Colour .	Positive
6.	Terpenoids	A reddish brown ring formation	Positive

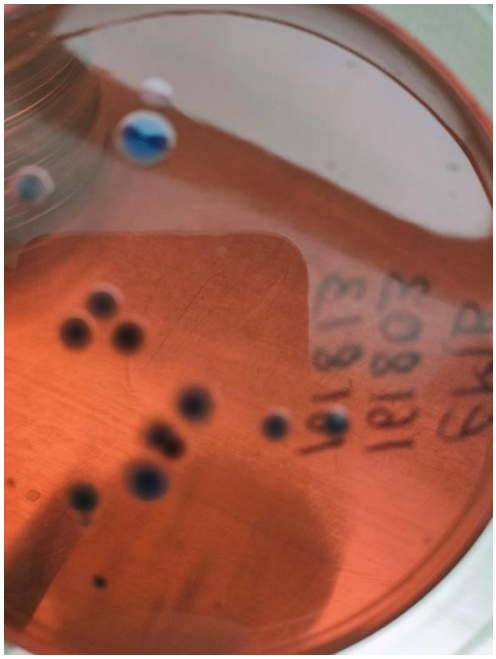


Acidity

Mineral Acidity

S NO.	Volume of sample (mL)	Burette reading (mL)		Volume of NaOH (mL)
		Initial	Final	
1.	50	0	0.4	0.4
2.	50	0	0.3	0.3
3.	50	0	0.2	0.2
4.	50	0	0.2	0.2

Bacterial Colonies on EMB media



- **CALCULATION OF TDS(Total Dissolved Solids)**

Initial weight of crucibles

Final weight of crucibles

As the TDS is calculated as final weight substracted from initial weight so

1. 19.687g-19.684g=0.003g
2. 21.808g-21.801g=0.007g

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