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PG - Department of Microbiology

(स्वायत्त) कोल्हापूर

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Place: Kolhapur

Date: 13/05/2024

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Place: Kolhapur
Date: 13/03/2024

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Place: Kolhapur

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Place: Kolhapur

Date:

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Place: Kolhapur
Date: 13/5/2024

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CERTIFICATE OF "ON JOB TRAINING"

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Place: Kolhapur
Date: 13/6/24

Examiner

OJT In charge

DEPARTMENT OF MICROBIOLOGY
VIVEKANAND COLLEGE,KOLHAPUR
(EMPOWERED AUTONOMOUS)

A

On Job Training report

In Collaboration with

Infinite Biotech Research and Analytics Institute, Sangli.

And

PG Department of Microbiology

Vivekananda College, Kolhapur (Empowered Autonomous)

By

Vishal Bapu Gore

M. Sc. Microbiology

Part I Semester I

Under the Guidance of

Dr. S. D. Mali

PG Department of Microbiology

DECLARATION

I the undersigned hereby declare that the On Job Training Report in Collaboration with **Infinite Biotech Research Institute**

, **Sangli** and PG Department of Microbiology,Vivekanand College, Kolhapur (Empowered Autonomous) is an original work done by me under the guidance of Dr. S. D. Mali PG Department of Microbiology, Vivekanand College, Kolhapur (Empowered Autonomous). The matter included in this report is not a reproduction from any other sources.

I also hereby declare that this project has not been submitted to any time to any other university or institution for the award of any degree or diploma.

Date:

Place: Kolhapur **Vishal Bapu Gore**

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At this juncture where the herculean task is nearing its pinnacle, science deems it a pleasure to look back and acknowledge efforts and support of all kith and kin that helped with zeal to turn a distant dream of an industrial training into reality.

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Also, we sincerely thank our parents for helping us in all aspects to complete the project work. Finally, we would like to appreciate our friends, colleagues for their direct and indirect contribution.

Date:

Place: Kolhapur **Vishal Bapu Gore**

INTERNSHIP UNDERTAKING

1. Student Name	Vishal Bapu Gore
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6. Aadhar	595180302511
7. PAN	-
8. Overall GPA	-
9. Internship Area (Company/Institute)	Infinite Biotech Research Institute, Sangli.

I confirm that I agree with the terms, conditions, and requirements of the Internship Policy

Student Signature:

Date:

I confirm that the student has attended the internship orientation and has met all paperwork and process requirements to participate in the internship program, and has received approval from his/her mentor.

Sign of Department Faculty Coordinator

Date

INDEX

Introduction

Infinite Biotech Institute of Research and Analytics established in 2022 with aim to provide innovators with necessary practical skills required for the culturing of animal cells, in vitro maintenance of cells. Students will be understanding the role of cells in the environment, and various applications. This lab focuses on various research activities including basic cell lines and cancer cell lines. Infinite Biotech creates ideas, inspires minds to solve problems, and addresses global challenges. We conduct fundamental and applied research that helps the world to better understand fundamentals of Animal Tissue Culture, Biotechnology, Microbiology and Materials Science. We translate that knowledge into new and unique ideas and innovations, processes, and technologies that advance the nation's energy and economic competitiveness and enhance national innovations.

Extraction of Coriandrum sativum.

Extraction is a common technique used in organic chemistry to isolate a target compound from biological samples such as cells, tissues, or fluids. In the two main types of extraction, which are liquid-liquid extraction and liquid-solid extraction, the separation is based on solubility. After extraction is complete the solvent can be removed and the desired product collected. The process of separating or extracting particular compounds or components, is referred to as extraction.

Steps involved in extraction process

1) Find the source-

Eg. Plant (Coriandrum sativum)

2) Selection of plant part-

Eg. leaves part.

3) Collection -

Collect Coriandrum plant leaves.

4) Washing-

Tap water is used for washing.

5) Cutting of leaves.

6) Drying-

In Hot Air Oven at 40℃.

7) Powdering-

Dry leaves can be powdered by use of mortar.

8) Methods of extraction-

1. Soxhlet method.
2. Maceration.

Extraction of Coriandrum sativum by Soxhlet Method

Soxhlet Method was developed by Franz von Soxhlet, in 1879. Soxhlet extraction is a Continuous solid liquid extraction in which active phytoconstituents are concentrated by use of organic solvents in Soxhlet apparatus.

Aim:

To prepare ethanolic extract of Coriandrum sativum using Soxhlet Method.

Principle:

The Soxhlet extraction method uses a small amount of solvent and is very cost-effective. The Soxhlet extraction uses the solvent reflux and siphon principle to continuously extract the solid matter by pure solvent, which saves the solvent extraction efficiency and high efficiency. The solid sample is placed on a thimble-shaped filter paper, positioned into the Soxhlet extractor, and the device is assembled. The solvent is added to the solvent reservoir flask and mounted onto a heating mantle. After heating, the condensed vapors of the solvent come in contact with the sample powder, and the soluble part of the powder gets mixed with the solvent for extraction. When the solvent surface exceeds the maximum height of the siphon, the solvent containing the extract is siphoned back. The flask is repeated, extracting a portion of the material each time so that the solid material is constantly used as a pure solvent and the extracted material is concentrated in the flask.

Procedure:

1. Assemble the Soxhlet apparatus according to the standard assembly.

2. Prepare the powder column with the help of filter paper. Pour 5g Coriandrum sativum powder into the paper column. Add 300 ml solvent in thimble for dissolving the powder.

3. Start the heating mantle initially with 10°C & after 15-20 mins increase temperature up to the boiling point of the solvent used.

4. Run approximately 6 cycles of the Soxhlet for proper extraction of phytochemicals.

Fig. Extraction of Coriandrum sativum by Soxhlet Method.

Result:

Extraction of given plant Coriandrum Sativum by using Soxhlet apparatus was performed by using 5 gm of powder of plant Coriandrum Sativum into extraction bag and bag was inserted into extraction column and then organic solvent like ethanol was added. After starting the heating mental the apparatus starts working and produce a plant sample after 6 cycle extract of given of Soxhlet apparatus.

Conclusion:

Soxhlet Method can be used for extraction of plant material. Phytochemicals can be extracted in their active form by use of organic solvents having low boiling point.

Extraction of Coriandrum sativum by Maceration Method

A biological process in which the Phytoconstituents are highly concentrated with gradual shaking is referred as maceration. Maceration process is influenced by numerous factors such as temperature, duration, solvent used, etc. It is an easy and popular technique that uses the concepts of diffusion and solubility to extract active ingredients.

Aim:

To Prepare the extract of given plant material using Maceration method.

Principle:

Maceration is a method of extraction where plant material is soaked in a solvent, typically a liquid like water or alcohol, to extract desired compounds. The principle involves allowing the solvent to penetrate the plant material, facilitating the dissolution of soluble compounds. This process occurs over an extended period, allowing for a comprehensive extraction. After maceration, the solvent is separated, leaving behind the extracted substances, often in the form of a liquid extract.

Procedure:

1. Weigh 10 gm Coriandrum sativum powder and dissolve it in 100 ml Distilled water. Cover the flask with the help of cotton and aluminium foil.
2. Make sure the Powder is totally dissolved into the water.
3. Incubate the flask at room temperature for 2-3 days in VDRL, Shaker.
4. Once the separated layer between Extract and Distilled water is observed after 2 days filter the extract with the help of filter paper or muslin cloth.
5. Collect the filtrate and evaporate it with help of boiling water bath.
6. After successful evaporation collect the extract in closed container and store it at 4°C for further use.

Fig. Extraction of Coriandrum sativum by Maceration Method using VDRL shaker.

Result:

Extraction of plant Coriandrum sativum by maceration was performed by using 5gm powder Coriandrum sativum of given plant sample dissolved it in 100 ml distilled water and covered it in the flask by using cotton and aluminium foil. After preparing the flask, the Flask were placed on VDRL shaker for 2 to 3 days to separate out two layers real extract and crude extract by using Filteration.

Conclusion:

In Maceration method, shaking and its duration time had a positive effect on yield of the extract. As compared to other method maceration method is very simple.

Phytochemical Analysis

Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections, and also consumption by insects and other animals. The name comes from Greek φυτόν (phyton) 'plant'. Some phytochemicals have been used as poisons and others as traditional medicine.As a term, phytochemicals is generally used to describe plant compounds that are under research with unestablished effects on health, and are not essential nutrients.

Two types of phytochemical analysis are performed.

1) Qualitative analysis

Qualitative analysis is performed to know presence or absence of phytoconstituents.

2) Quantitative analysis

Quantitative analysis is used to know quantity of the phytoconstituents present in extract.

Phytochemical Analysis:

Test for Glycosides:

1. **Keller Killani test:**

The test solution with few drops of glacial acetic acid 2ml of ferric chloride solution and concentrated sulphuric acid is added from side of test tube which show separation between two layers, lower layer shows reddish brown upper layer turns bluish green in colour.

2. **Raymond's test:**

Test solution treated with Dinitrobenzene in hot methanolic alkali gives violet colour.

3. **Legal's test:**

The test solution treated with 1ml pyridine and 1ml sodium nitroprusside gives pink to red colour appear.

Test for Alkaloids:

1. **Mayer's test :**
   Test solution treated with Mayer's reagent (potassium mercuric iodide) gives cream colour precipitate.

2. **Wagner's test:**
   The acidic test solution treated with Wagner's reagent (iodine in potassium iodide) gives brown precipitate.

3. **Hager's test:**
   The acetic acid test solution treated with Hager's reagent gives yellow precipitate.

Test for Flavonoids:

1. **Ferric chloride test:**
   The test solution with few drops of ferric chloride solution shows intense green colour.

2. **Shinoda test:**
   Test solution with few fragments of magnesium ribbon and concentrated hydrochloric Acid shows pink to magenta red colour.

3. **Zinc hydrochloric acid reduction test:**
   Test solution with zinc dust and few drops of hydrochloric acid shows magenta red color.

1. **Alkaline reagent test:**
   Test solution when treated with sodium hydroxide solution shows increase in intensity of yellow colour which becomes colorless on addition of few drops of dilute acid.

2. **Lead acetate solution test:**
   Test solution with few drops of lead acetate solution gives yellow precipitate.

Test for Steroids:

1. **Chloroform test:**

   The crude plant extract (1mg) was taken in the test tube and dissolved with chloroform (1ml) then added equal volume of concentrated sulphuric acid to the test tube by sides. The upper layer in test tube was turns red and sulphuric acid layer showed yellow with green fluorescence. It showed presence of steroid.

2. **Salkowski's test:**

   The second portion of solution above was mixed with concentrated sulphuric acid carefully so that acid formed a lower layer and interface was observed for reddish brown colour.

Test for Phenol:

1. **Ferric chloride test:**

   A small amount of ethanolic extract was taken with 3ml of water in test tube and 1to 2 drop of iron 3 chloride (FeCl3) was added. A blue, green, red or purple colour is positive test.

Test for Triterpenoids:

1. **Salkowski's test-**

When few drops of concentrated sulphuric acid are added to the test solution, shaken and allowed to stand, lower turn, red indicating the presence of Triterpenoids.

2. **Liebermann Burchard test-**

The test solution treated with few drops of acetic anhydride and mixed well when concentrated Sulphuric acid is added from the sides of the test tube, it shows a brown ring at the junction of the two layer & the upper layer turns green.

Test for Saponins:

1. Foam test-

Sample when mixed with water & shaken show the formation of foam which is stable at least for 15 min.

2. Haemolysis test-

12 ml of 18% L/V sodium chloride in two tubes wer taken. To one test tube distilled water & to the 'other test tube 2 ml of filtrate were added and then few drops of blood was added to both the tuber. Mixed and observed the Haemolysis under microscope.

3. Raymond's test-

The solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

Test for Carbohydrates:

1. Molisch's test :

Test solution with few drops of Molisch's reagent and 2ml of concentrated sulphuric acid added slowly from side of test tube shows purple ring at junction of liquid.

2. Barfoed's test:

Test solution treated with barfoed's reagent boiling on water bath shows brick red precipitate.

3. Benedicts test:

Test solution treated with benedict's reagent and boiling on water bath shows reddish brown precipitate.

Test for Proteins:

1. **Millon's test :**
   Test solution treated with millon's reagent and heated on water bath protein is stained yellow on warming.

2. **Xanthoproteic test:**
   Test solution treated with concentrated nitric acid and boiling gives yellow precipitate.

3. **Biuret test:**
   Test solution treated with 40% sodium hydroxide and dilute copper sulphate solution gives blue color.

4. **Ninhydrin test:**
   Test solution treated with ninhydrin reagent gives blue colour.

Test for Starch:

1. **Starch reagent test:**
   1ml of extract was added into 10ml of NaCl solution.After heating,starch reagent was added a blue grayish colour is positive test for presence of starch.

Test for Tannins:

1. **Gelatin test:**
   Plant extract is dissolved in 5ml of distilled water and 1%gelatin solution and 10%NaCl.Reaction gives white precipitate.

2. **NaOH test:**
   4ml of 10% NaOH into 0.4ml of extract and shaken well and formation of emulsion.

Test for Flavonoids:

1. **Ferric chloride test-**

   The test solution with few drops of ferric chloride solution shows intense the color.

2. **Shinoda test-**

   Test solution with few fragments of magnesium ribbon and concentrated hydrochloric acid Show pink to magenta red color.

3. **Zinc-Hydrochloric acid-reduction test-**

   Test solution with zinc dust and few drops of hydrochloric acid shows magenta red color.

4. **Alkaline reagent test-**

   Test solution when treated with sodium hydroxide solution shows increase in the intensity color which becomes colorless on few drops of dilute acid.

5. **Lead acetate solution test-**

   Test solution with few drops of lead acetate (solution (10% w/v) gives yellow precipitate.

Sr.no	Name of component	Name of Test	Observation	Present or Absent
1.	Glycosides	1)Killer killani test	Brown and green colour present	Present
		2)Raymond's test	No colour formed	Absent
		3) Legal's test	Red colour present	Present
2.	Alkaloids	1)Mayers test	No precipitate formed	Absent
3.	Flavonoids	1)Ferric chloride test	Intense green colour formed	Positive
		2)Shinoda test	No colour formation	Absent
		3)Zinc hydrochloric acid reduction test	No colour formation	Absent
		4)Alkaline reagent test		Present
		5)Lead acetate test	Yellow precipitate formed	Present
4.	Steroids	1)Salkowski's test	Reddish brown colour formed	Present
		2)Chloroform test		Present
5.	Phenol	1)Ferric chloride test	Green colour formed	Present
6.	Triterpenoids	1)Salkowski's test	Red colour formed	Present
		2) Liberman Burchard test	Brown ring appears	Present
7.	Saponins	1)Raymonds test	No colour formation	Absent
8.	Carbohydrates	1)Molisch test	Purple ring appeared	Present
		2)Benedict's test	No precipitation	Absent
9.	Proteins	1)Millons test	No colour formation	Absent
		2)Xantho proteic test	Yellow precipitate formed	Present
		3)Biuret test	Blue colour appears	Present
		4)Ninhydrin test	No colour formation	Absent
10.	Starch	1)Starch reagent test	No colour formation	Absent
11.	Tannin	1)Gelatin test	No precipitation	Absent
		2)NaOH test	Formation of emulsion	Present
12.	Flavonoids	1) Ferric chloride test	Green colour formed	Present
		2)Shinoda test	No colour formation	Absent
		3)Zinc hydrochloric acid reduction test	No colour formation	Absent
		4)Alkaline reagent test		Present
		5)Lead acetate reduction test	Yellow precipitate formed	Present

Fig: Phytochemical Analysis of Plant Coriandrum sativum.

Conclusion:

In given plant sample, Coriandrum sativum contains various phytochemicals such As Glycosides, Flavonoids, Steroids, Phenols, Triterpenoids, Carbohydrates, Protein, Flavonol etc.

Thus phytochemical analysis of Coriandrum sativum by qualitative method was performed.

QUANTITATIVE ANALYSES OF PHYTOCONSTITUENTS

Estimation of Total Phenol by FCR Method using Plant Coriandrum sativum.

Aim:

To estimate the Total Phenolic content by FCR method.

Principle:

The estimation of total phenols typically involves using a colorimetric method, such as the Folin-Ciocalteu assay. In this method, phenolic compounds react with the Folin-Ciocalteu reagent, forming a blue complex. The intensity of the color is proportional to the concentration of phenols and can be measured spectrophotometrically. Calibration with known standards helps quantify the total phenolic content in the sample.

Reagent preparation:

1. Plant extract.

2. Folin-Ciocalteu reagent.

3. 10% Sodium carbonate.

4. Standard-Gallic acid.

Procedure:

1) Reaction mixture contains 1ml of plant extract and 9ml of distilled water.

2) Add 1ml of Folin reagent in above reaction mixture.

3) Incubate the flasks for 5 min at room temperature.

4) After successful incubation add 10% 10 ml sodium carbonate in above reaction mixture.

5) Incubate the flasks for 90 min at RT.

6) After successful incubation measure the absorbance at 569nm.

7) Plot the standard curve on graph.

Fig.Estimation of Total Phenol : Standard used as Gallic Acid.

Fig. Estimation of Total Phenol: Test sample

FORMULA:

% OF INHIBITION =O.D. OF CONTROL-O.D. OF TEST/O.D. OF CONTROL×100

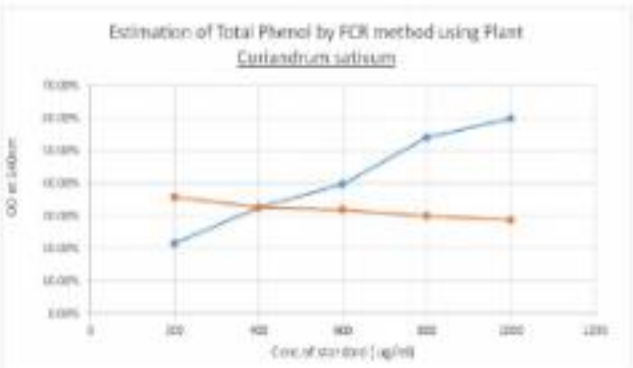
OBSERVATION TABLE:

STANDARD:-

Concentration (microgram/ml)	O.D. at 560nm
200	0.14
400	0.26
600	0.29
800	0.30
1000	0.39

TEST:-

Concentration (microgram/ml)	O.D. at 560nm
200	0.08
400	0.10
600	0.16
800	0.27
1000	0.30



Result :

Estimation of total phenol by FCR method was performed using 1 ml of plant extract in 9ml distilled water and 1 ml Fc reagent was added in above reaction and incubated at RT for 5 mins.

After incubation, 7% of 10 ml sodium carbonate was added and incubated for 90 minutes at RT and measured the absorbance of reaction mixture at 540 nm.

Estimation of Total Flavonoids by AlCl3 of Plant Coriandrum sativum.

Aim:

To estimate total flavonoid content by AlCl3 method.

Principle:

The estimation of total flavonoids using aluminum chloride (AlCl3) involves a colorimetric method. Flavonoids react with AlCl3 to form a complex with a yellow color. The intensity of this color is proportional to the concentration of flavonoids in the sample. UV-Vis spectrophotometry is commonly used to measure the absorbance of the complex, and the results are compared against a standard curve generated using known concentrations of a flavonoid standard. This method is widely employed for assessing the total flavonoid content in plant extracts or other samples.

Reagent preparation:

1) Plant extract
2) 10% sodium nitrite.
3) 5% Aluminium chloride.
4) 1M NaOH
5) Standard-Quercetin

Procedure:

1) The reaction mixture contains 1 ml of plant extract and 4 ml of distilled water.
2) Further add 0.3ml of 5% AlCL3 and 0.3ml of 10% sodium nitrite.
3) Incubate the reaction mixture for 5 min. at room temperature.
4) Further add 2 molar 2ml sodium nitroxide in an above reaction mixture.
5) Incubate the reaction mixture for 30 min. at room temperature
6) Measure the absorbance at 560nm.

Observation table:

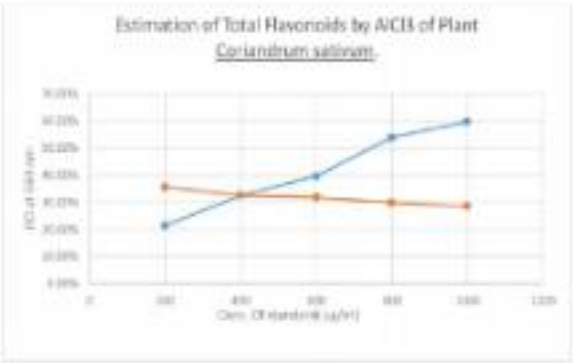
Standard:

Concentration (microgram/ml)	O.D. at 560nm
200	0.08
400	0.10
600	0.16
800	0.27
1000	0.30

Test:

Concentration (microgram/ml)	O.D. at 560nm
200	0.22
400	0.43
600	0.70
800	0.73
1000	0.84

Fig. Estimation of Total Flavonoids by AlCl3 of Plant Coriandrum sativum



Result:

Estimation of total Flavonoids by Aluminium Chloride method was performed using 1 ml plant extract and 4 ml of distilled water then 0.3 ml of 5% AlCl3 was added following 0.3ml of 10% sodium hydroxide.The reaction mixture was incubated for 5 minutes at room temperature.

After Incubation 2M ml sodium hydroxide was added and incubated the reaction mixture for 30 minutes at room temperature then measured the absorbance of the reaction mixture at 590nm.

Biomedical Application

Estimation of Anti-Oxidant Assay by DPPH method using Plant Coriandrum sativum.

Antioxidants are compounds that inhibit oxidation, a chemical reaction that can produce free radicals. Antioxidation leads to degradation of organic compounds, including living matter. Antioxidants are frequently added to industrial products, such as polymers, fuels, and lubricants, to extend their useable lifetimes.

Aim:

To estimate the Anti-oxidant activity by DPPH method.

Principle:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is based on the principle of color change as a result of the reduction of the stable free radical DPPH by antioxidants. The DPPH radical is deep violet, and when it encounters antioxidants in a sample, it accepts electrons, leading to a reduction in its color intensity.

The reaction can be represented as follows:

DPPH+ Antioxidant→DPPH-H+Reduced Antioxidant

The decrease in absorbance at a specific wavelength is measured using a spectrophotometer, and the results are compared against a standard curve or known concentrations of antioxidants to quantify the antioxidant capacity of the sample.

Reagent preparation:

1) Plant Extract
2) DPPH reagent
3) Standard-Ascorbic Acid.

Procedure:

1) The reaction mixture contains 1 ml of DPPH and 1 ml of plant extract or standard (ascorbic acid or Vitamin C).
2) Incubate the reaction mixture at 37°C for 30 minutes in dark condition for stability.
3) Measure the absorbance of reaction mixture at 510 nm.

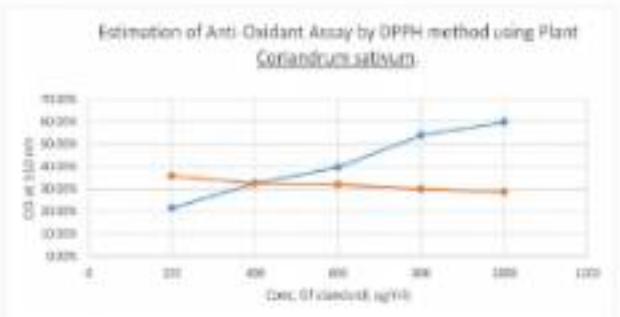
FORMULA:

% OF INHIBITION = O.D. OF CONTROL-O.D. OF SAMPLE/O.D. OF CONTROL*100

Fig. Estimation of Anti-Oxidant Assay by DPPH method using Plant Coriandrum sativum.

OBSERVATION TABLE:

Concentration (mg/ml)	O.D. AT 510nm		% OF INHIBITION	
	STANDARD	TEST	STANDARD	TEST
2	0.02	0.42	98.09%	72.34%
4	0.03	0.53	98.03%	65.35%
6	0.04	0.97	97.38%	36.60%
8	0.07	1.03	95.42%	32.67%
10	0.10	1.23	93.46%	19.60%



Result:

Estimation of antioxidant activity of plant Coriandrum sativum by DPPH method was performed using 1 ml of DPPH and 1 ml of plant extract and incubated it at 37° Celsius for 30 minutes in dark condition .

After Incubation, measure the absorbance of reaction mixture at 510 nm.

Conclusion:

The conclusion for antioxidant activity by the DPPH (2,2-diphenyl-1- picrylhydrazyl) method is determined by measuring the ability of a substance to scavenge the DPPH free radical. The DPPH assay is widely used to evaluate the antioxidant potential of natural and synthetic compounds. The conclusion drawn from this method depends on the percentage of DPPH radical scavenging activity exhibited by the test sample. A higher percentage of inhibition indicates stronger antioxidant activity, while a lower percentage suggests weaker antioxidant potential.

Anti-Inflammatory assay by protein denaturation method using Plant Coriandrum sativum.

Aim:

To evaluate the anti-inflammatory activity of the given extract by protein denaturation method.

Principle:

The protein denaturation method is often used to assess anti-inflammatory activity. In this assay, the principle revolves around the prevention of protein denaturation, which is a key step in the inflammatory process. Proteins denature during inflammation, leading to structural changes.In the anti-inflammatory assay, a sample is tested to determine its ability to inhibit the denaturation of a protein, often using a standard protein like albumin. The degree of protein denaturation is assessed by measuring changes in turbidity or absorbance. A lower absorbance or turbidity in the presence of the sample indicates its potential anti-inflammatory activity, as it suggests protection against protein denaturation that occurs during inflammation.

Reagents preparation:

1) Egg albumin & PBS (Phosphate buffer saline)

2) Standard-Diclofenac sodium

Procedure:

1) The reaction Mixture contains 0.4 ml of egg albumin (collected from fresh hen's egg),5.6% phosphate-buffered saline (PBS),

2) Further add 1ml plant extract& Standard- Diclofenac sodium.

3)Incubate the reaction mixture for 30mins at 70°C

4) After successful incubation measure the absorbance of reaction mixture at 510nm

Formula:

% OF INHIBITION =O.D. OF CONTROL-O.D. OF TEST/O.D. OF CONTROL * 100

Fig. Anti-Inflammatory assay by protein denaturation method using Plant Coriandrum sativum: Test sample.

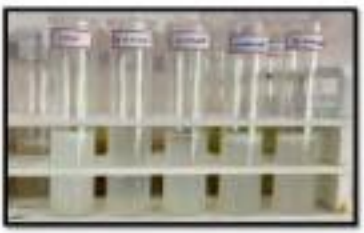
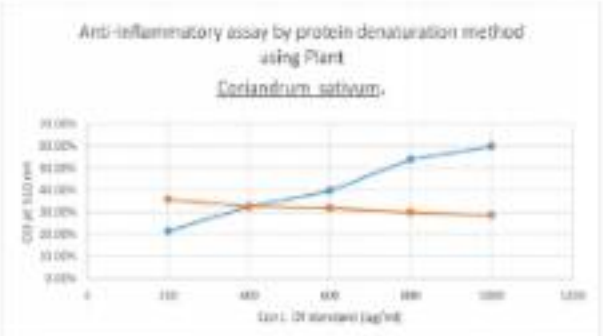


Fig. Anti-Inflammatory assay by protein denaturation method using Plant Coriandrum sativum: Standard used is Diclofenac sodium.

OBSERVATION TABLE:

Concentration (Mg/ml)	O.D. at 510nm		% of inhibition	
	standard	test	Standard	Test
200	0.98	0.90	20.56%	27.41%
400	0.86	1.02	30.64%	17.74%
600	0.98	1.36	20.56%	9.67%
800	0.93	1.52	25%	22.58%
1000	1.11	1.99	10.48%	60.48%



Result:

Estimation of anti-inflammatory activity of Coriandrum sativum by protein denaturation method was performed using 0.4 ml of fresh egg albumin and 5.6 ml PBS (Phosphate Buffer Saline) and 1 ml plant extract was added and incubated it for 30 minutes at 70°Celsius in water bath.

After incubation, the absorbance of reaction mixture was measured at 510 nm and percentage of inhibition was calculated.

Conclusion:

Protein denaturation is a process in which proteins lose its structure by application of external stress and it is the reason for inflammatory disease. The ability of flavonoid-rich extract of Coriandrum sativum shows anti-inflammatory activity and can be used in formulations against inflammation.

Anti-diabetic Assay by Alpha- Amylase Method using Plant Coriandrum sativum.

An antidiabetic assay using the alpha-amylase method is designed to evaluate the potential of substances to inhibit the enzyme alpha-amylase, crucial in carbohydrate digestion. Inhibition of alpha-amylase can help regulate glucose levels, making this assay valuable in identifying compounds with anti-diabetic properties. The assessment often involves measuring the inhibition's impact on the breakdown of starch, providing insights into a substance's ability to modulate postprandial glucose levels, a key aspect in diabetes management.

Aim:

To perform anti- diabetic activity by alpha amylase method.

Principle:

In the alpha-amylase inhibition assay, the principle lies in assessing the ability of a substance to inhibit the activity of the enzyme alpha-amylase. Alpha-amylase is involved in the breakdown of complex carbohydrates into simpler sugars, including glucose. Inhibition of this enzyme can help regulate postprandial glucose levels, making it relevant to anti-diabetic research.The degree of inhibition is often measured by assessing the reduced formation of reducing sugars, typically through colorimetric or spectrophotometric methods. A lower absorbance or color development indicates a higher level of inhibition, suggesting potential anti-diabetic properties by regulating carbohydrate digestion.

Reagent preparation:

1) Plant extract
2) DNSA
3) Test sample 0.5%Alpha amylase
4) 1% starch

Procedure:

1) The reaction mixture contains 0.5ml of Plant / std. - Acarbose, & 0.5 ml of α-amylase (0.5%)
2) further add 1% starch to and above reaction mixture.
3) Incubate the reaction mixture at RT. for 30 min.
4) Further add reaction 2 ml DNSA in an above reaction mixture.
5) Incubate the reaction mixture for 15 min in boiling water bath. 100°C
6) After successful Incubation Measure the absorbance of the reaction mixture at 560nm.

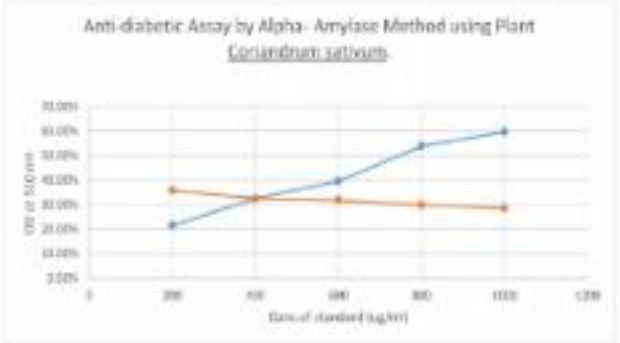
Formula:

% OF INHIBITION =O.D. OF CONTROL-O.D. OF TEST/O.D. OF CONTROL×100

Fig. Anti-diabetic Assay by Alpha- Amylase Method using Plant Coriandrum sativum: Test sample.

Observation Table:

Concentration (ug/ml)	OD at 560nm		% of Inhibition	
	standard	Sample	Standard	Sample
200	1.21	0.99	21.42%	35.71%
400	1.04	1.03	32.46%	32.46%
600	0.93	1.05	39.61%	31.81%
800	0.71	1.08	53.89%	29.87%
1000	0.62	1.16	59.74%	28.57%



Result:

Estimation of antidiabetic activity of Coriandrum sativum by alpha amylase method was performed using 0.5 ml of plant extract added in 0.5 ml 0.5% of alpha amylase further 0.5ml 1% starch was added and incubated it for 30 minutes at room temperature.

After incubation 2ml DNSA was added in reaction mixture and again incubated for 15 minutes in a boiling water bath and then measured the absorbance of reaction mixture at 560nm and also percentage of inhibition was calculated.

Conclusion:

The anti-diabetic assay using the alpha amylase method is a valuable technique for evaluating the potential of various compounds or extracts in managing diabetes. This method measures the enzymatic activity of alpha amylase, which plays a crucial role in the breakdown of carbohydrates, and its inhibition is known to have a positive impact on blood glucose levels.

Anti-Helmenthic Assay of Coriandrum sativum.

An antihelminthic assay is a laboratory test conducted to evaluate the effectiveness of substances in combating parasitic worm infections. This assay typically involves exposing the worms to the test substance and assessing parameters such as viability, motility, and reproductive capacity. The results aid in identifying potential agents with anti-parasitic properties, crucial for developing treatments against helminthic infections in both animals and humans.

Aim :

To estimate the anti-helmenthic activity of Coriandrum sativum.

Principle :

The principle of an antihelminthic assay involves evaluating the ability of a substance to inhibit the survival, growth, or reproduction of parasitic worms, known as helminths. This assay is designed to identify potential agents with anti-parasitic properties. Typically, the process includes exposing the worms to the test substance and assessing parameters like mortality, motility, and reproductive capabilities. The degree of inhibition or damage to the helminths provides valuable insights into the efficacy of the tested compound as a potential treatment for parasitic worm infections.

Requests :

1) Plant extract
2) Standard – Albendazole

Procedure:

1) Take 2 petri plates.Clean the petri plates and air dry.
2) Collect the earthworm and wash them with the help of water to remove the unwanted soil dust.
3) Take 5 earthworms in each petri plates and close with the help of petri lid.
4) Load drug(Albendazole) and sample in different petri plates and note the time of paralysis and time of death.

Fig. Anti-Helmenthic Assay of Coriandrum sativum.

Observation:

	Standard	**Plant extract**
Paralysis Time	6.00 mins	11.25 mins
Death Time	8.00 mins	11.30 mins

Result:

Estimation of anthelmintic activity of plant Coriandrum sativum was performed by using 2 petri plates ,cleaned and dried it. After selecting the earthworms clean them, then earthworms were distributed in each petri plate. In one petriplate sample 20 ml was added and in another one standard (Albendazole) 20 ml was added. Then time of paralysis and time of death of Earthworms was measured.

As per time taken by earthworms for paralysis in sample was 11.25 minutes and time for death was 11.30 minutes and for standard time for paralysis was 6 minutes and time for death was 8 minutes.

Conclusion:

Plant extract of Coriandrum sativum shows effective activity against earthworm. Thus can be used to treat diseases caused by parasitic intestinal.

Anti-Bacterial Activity by Well Diffusion Method using Plant Coriandrum sativum.

The term "anti-bacterial activity" refers to the ability of a substance to inhibit the growth or kill bacteria. This activity is of significant interest in various fields, including medicine, food preservation, and agriculture. There are numerous natural and synthetic compounds that exhibit antibacterial activity, and understanding their mechanisms and effectiveness is crucial for developing new antibiotics, disinfectants, and antimicrobial agents.

Aim :

To study the Anti-Bacterial activity by well diffusion method.

Principle :

The well diffusion method assesses antibacterial activity by introducing a substance into wells on an agar plate inoculated with bacteria. The principle involves the diffusion of the substance through the agar, creating a concentration gradient. The zone around the well where bacterial growth is inhibited indicates the effectiveness of the substance against the tested bacteria. A larger zone of inhibition suggests stronger antibacterial activity, providing valuable information about the substance's potential as an antibacterial agent.

Reagents :

1) Plant extract
2) Standard – Streptomycin

Procedure :

1) Prepare the nutrient agar media and autoclave it.
2) Pour the media into respective petri dishes and allowed to solidified it.
3) Prepare the bacterial inoculum and spread it on the top of nutrient agar media.
4) After preparation of plates make well with the help of cork borer.
5) Inoculate the sample into particular wells.
6) After inoculation incubate the plates at 37°C for 24 hrs.
7) After successful incubation measure zone of inhibition.

Fig. Anti-Bacterial Activity by Well Diffusion Method using Plant Coriandrum sativum.

Observation:

Concentration of standard	Concentration of sample	Inhibition zone of standard	Inhibition zone of sample
100 ul	100 ul	20 mm	17 mm

Result :

Estimation of antibacterial Activity of plant Coriandrum sativum by well diffusion method was performed by preparing sterile nutrient agar medium and prepare the bacterial inoculum and spread on the plates. After spreading, the wells are prepared by using cork borer and in that wells the sample and standard (Streptomycin) were inoculated and incubated the plates at 37° Celsius for 24 hours.After successful incubation, the zone of inhibition were measured.

As per the zone of inhibition the zone of sample was 17 mm and zone of standard was 20 mm. In each well 100 microliter sample and standard was inoculated.

Conclusion :

In this study antibacterial activities of Coriandrum sativum assessed by well diffusion method.

Anti-Fungal Activity by Well Diffusion Method using Plant Coriandrum sativum.

Antifungal activity refers to the ability of a substance to inhibit or kill the growth of fungi. This activity is of particular interest in various fields, including medicine, agriculture, and industry, where fungal infections or contaminants can have significant implications. Antifungal agents can be natural or synthetic compounds that target specific fungal structures or functions.

Aim :

To study the Anti-Fungal activity by well diffusion method.

Principle :

The principle of antifungal activity by the well diffusion method involves assessing the ability of a substance to inhibit fungal growth. In this method, the substance is placed in wells on an agar plate inoculated with fungal cultures. As the substance diffuses into the agar, it creates a concentration gradient, leading to inhibition of fungal growth around the well. The size of the resulting zone of inhibition is indicative of the substance's effectiveness against the tested fungi, providing valuable information about its antifungal activity.

Reagents :

1) Plant extract
2) Standard – Miconazole

Procedure:

1) Prepare the Sabouraud's Agar media and autoclave it.
2) Pour the media into respective petri dishes and allow to solidify it.
3) Prepare the inoculum and spread it on the top of Sabouraud's agar plate media.
4) After preparation of plates make the well with the help of cork borrer.
5) Inoculate the sample in particular wells.
6) After the inoculation incubate the plates at room temperature for 24 hrs.

Fig. Anti-Fungal Activity by Well Diffusion Method using Plant Coriandrum sativum.

Observation:

Concentration of standard	Concentration of sample	Inhibition zone of standard	Inhibition zone of sample
100 ml	100 ml	22 mm	15 mm

Result :

Estimation of antifungal activity of plant Coriandrum sativum by using well diffusion method was performed by preparing the sterile Sabourad's agar medium and the fungal inoculum.Then inoculum was spread on the plates and after spreading the wells are prepared by using cork borer and in that wells the sample 100 microliter and standard (Miconazole) 100 microliter were inoculated and incubated the plates at room temperature for the 2-3 days and after the successful incubation, the zone of inhibition was measured.

As per zone of inhibition the zone of sample was 15 mm and zone of inhibition for the standard was 22 mm

Conclusion :

In this study antifungal activities of Coriandrum sativum assessed by well diffusion method.