SONOPANT DANDEKAR ARTS, V.S. APTE COMMERCE AND M.H. MEHTA SCIENCE COLLEGE, PALGHAR

Department of Biotechnology

PROJECT REPORT

MASTER OF SCIENCE -BIOTECHNOLOGY

Academic Year 2022-2023

Prepared by

Department of Biotechnology

Sonopant Dandekar Arts, V.S. Apte Commerce and M.H. Mehta Science College, Palghar

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Sonopant Dandekar Shikshan Mandali's
SONOPANT DANDEKAR ARTS, V.S. APTE COMMERCE AND M.H. MEHTA SCIENCE COLLEGE, PALGHAR
Palghar, Dist – Palghar, Pin – 401 404, E – Mail: sdsmcollege@yahoo.com

Department of Biotechnology

NOTICE

Date: 01st July 2023

Subject: Submission of Project Report

This is to inform you that all the **M.Sc. Part-II Biotechnology** students are required to submit the hard copy of your final project report by **7th July 2023**. All submissions should be made to the **Biotechnology Department** during office hours from **10:00 am to 02:00 pm**. Ensure your report is properly printed and bound.

Co-ordinator
Department of Biotechnology

(Dr. Kiran J.Save)
Principal

PRINCIPAL
Sonopant Dandekar Arts College,
V.S. Apte Commerce College &
M.H. Mehta Science College
PALGHAR (W.R.)
Dist. Palghar, Pin-401404

University of Mumbai

No. UG/ 05 of 2020-21

CIRCULAR:-

Attention of the Principals of the Affiliated Colleges, the Head of the University Departments and Directors of the recognized Institutions in Science & Technology Faculty is invited to the syllabus uploaded Academic Authority Unit which was accepted by the Academic Council at its meeting held on 27th February, 2015 vide item No.4.67 relating to the revised syllabus as per the (CBSGS) for the M.Sc.(Part-II) (Sem. III & IV) in Bio-Technology.

They are hereby informed that the recommendations made by the Ad-hoc Board of Studies in Bio-Technology at its meeting held on 6th May, 2020 and subsequently made by the Board of Deans at its meeting held on 20th July, 2020 have been accepted by the Academic Council at its meeting held on 23rd July, 2020 vide item No.4.104 and that in accordance therewith, the revised syllabus as per the (CBCS) of M.Sc. (Part II) (Sem. III & IV) in Bio-Technology has been brought into force with effect from the academic year 2020-21, accordingly. (The same is available on the University's website www.mu.ac.in).

(Dr. Vinod Patil)
I/c REGISTRAR

MUMBAI – 400 032
14th September, 2020

To

The Principals of the affiliated Colleges, the Head of the University Departments and Directors of the recognized Institutions in Science & Technology Faculty. (Circular No. UG/334 of 2017-18 dated 9th January, 2018.)

A.C/4.104/23/07/2020

****

No. UG/ 05 -A of 2020-21 MUMBAI-400 032 14th September, 2020

Copy forwarded with Compliments for information to:-

1) The I/c Dean, Faculty of Science & Technology.
2) The Chairman, Board of Studies in Bio-Technology.
3) The Director, Board of Examinations and Evaluation.
4) The Director, Board of Students Development.
5) The Co-ordinator, University Computerization Centre.

(Dr. Vinod Patil)
I/c REGISTRAR

AC_____

Item No. _____

Program : M.Sc. Biotechnology Course : M.Sc.Biotechnology Syllabus for Semester: III & IV

(Choice Based and Credit System with effect from the Academic year 2020-21)

Item No. _____

Cover Page

AC_____

UNIVERSITY OF MUMBAI

Syllabus for Approval

Sr. No.	Heading	Particulars
1	Title of the Course	M.Sc. (Biotechnology)
2	Eligibility for Admission	B.Sc. (Biotechnology)
3	Passing Marks	40%
4	Ordinances / Regulations (if any)	--
5	No. of Years / Semesters	2 years/ Four semesters
6	Level	P.G. / ~~U.G./ Diploma / Certificate~~ **(Strike out which is not applicable)**
7	Pattern	~~Yearly /~~ Semester **(Strike out which is not applicable)**
8	Status	~~New /~~ Revised **(Strike out which is not applicable)**
9		emented from From Academic Year

Date: Signature :

Name: **Dr. Anuradha Majumdar** (Dean, Science and Technology) **Dr. Archana Rath** (Chairperson, Ad Hoc BOS in Biotechnology)

PREAMBLE

In recent years, there has been a paradigm shift in education in terms of course content and learning outcomes. In keeping with it, the Faculty of Science & Technology, University of Mumbai, has taken a progressive step to align various programs under its purview with the current Higher Education Policy of the Government of India. The present M.Sc. Biotechnology Second Year (Semester III and IV) syllabus has been designed with the idea of incorporating outcome-based learning for fruitful engagement of learners. The syllabus has undergone several curriculum revision exercise based on the remodeled M.Sc. Biotechnology Curriculum, May 2017, Department of Biotechnology, Ministry of Science and Technology, Government of India. The revised syllabus is an outcome of several rounds of deliberations, discussions, feedback and multiple brainstorming sessions involving various contributors & stakeholders- academicians, researchers, industry experts and students. Course Objectives and Course Outcomes have been clearly defined for each paper in the syllabus to guide teachers in order to make learning process more effective. A lot of focus has been given in the syllabus to cover latest developments in the area of biotechnology and to equip students with necessary knowledge and skills. Relevant papers to make students industry ready have also been included. Attempts have been made to draft a robust, well defined syllabus keeping in view the best learning outcome which shall enable students to pursue high quality research or increase employability of the students. Online course component has been introduced in the curriculum in keeping with the digital initiatives of MHRD to provide good quality self-learning content through MOOCs under SWAYAM and allied platforms.

It is hoped that the revised syllabus shall serve its objective of promoting outcome-based learning to meet the changing needs of the biotechnology sector.

Dr. Anuradha Majumdar (Dean, Science and Technology)
Prof. Shivram Garje (Associate Dean, Science)
Dr. Archana Rath (Chairperson, Ad Hoc BOS in Biotechnology) Dr. Deepali Karkhanis (Member)
Dr. Sneha Panvalkar (Member)
Dr. Seema Kokitkar (Member)
Dr. Tara Menon (Member)
Dr. Jayaprada R. Chunduri (Member)
Dr. Bhupendra Pushkar (Member)
Dr. Rajesh C. Patil (Member)

MSc Biotechnology Course Structure

Semester III

Course code	Title	Theory /Practical	Marks	Credits	Nos of Lectures /week
PSBT 301	Applied virology and microbiology	Theory	100	4	1
PSBT 302	Environmental Biotechnology	Theory	100	4	1
PSBT 303	Biologics and Regulatory Affairs	Theory	100	4	1
PSBT 304	Molecular Enzymology and Enzyme Technology	Theory	100	4	1
PSBTP301	Practical I	Practical	50	2	4
PSBTP302	Practical II	Practical	50	2	4
PSBTP303	Practical III	Practical	50	2	4
PSBTP304	Practical IV	Practical	50	2	4
	TOTAL		**600**	**24**	

MSc Biotechnology Course Structure

Semester IV

Course code	Title	Theory /Practical	Marks	Credits	No. of Lectures/ week
PSBT 401	Nanobiotechnology	Theory	100	4	1
PSBT 402	OMICS & Systems Biology	Theory	100	4	1
PSBT 403	Drug Discovery & Clinical Study	Theory	100	4	1
PSBT 404	Scientific Writing & Food Biotechnology	Theory	100	4	1
PSBTP401	Practical I	Practical	50	2	4
PSBTP402	Practical II	Practical	50	2	4
PSBTP403 & PSBTP404	Practical III & IV	Practical	100	4	8
	TOTAL		**600**	**24**	

Teaching pattern:

One (01) Credit would be of thirty-forty (30-40) learning hours; of this, more than fifty per cent of the time will be spent on classroom instructions including practical as prescribed by the University. Rest of the time would be invested for assignments, projects, journal writing, case studies, library work, industrial visits, attending seminars/workshops, preparations for examinations etc. would be considered as notional hours. The present syllabus considers (60L as classroom teaching and 15 lectures as Notional hours/ paper). Each lecture duration would be for 60 min. The names of the reference books provided in the syllabus are for guidance purpose only. Students and faculty are encouraged to explore additional reference books, online lectures, videos, science journals for latest/ additional information.

Scheme of Examinations:

(a) Internal assessment of 40 marks per course per semester should be conducted. (b) External assessment of 60 marks per course per semester at the end of every semester (c) Practical examination of 200 marks should be conducted at the end of every semester.

A. Semester III- Theory -Internal assessment (40%) -40 marks

Sr No	Evaluation type	Marks
1.	Assignments that can include article writing, report writing, preparation of a review, on any topic selected from each paper OR PowerPoint presentation on a topic from the syllabus or related to the syllabus *Note: The student can submit only 2 assignments and give 2 PowerPoint presentations per semester. Respective college/department can decide the allocation.*	30
2.	**a.** Active participation in routine class instructional deliveries	05
	b Overall conduct as a responsible student, w.r.t manners, skill in articulation, leadership qualities demonstrated through organizing co curricular activities, etc.	05
	Total Marks	40

Semester IV- Theory -Internal assessment (40%) -40 marks

Sr. No	Evaluation type	Marks

1.	**For PAPER 1 & 2** Assignments that can include article writing, report writing, preparation of a review, on any topic selected from each paper OR PowerPoint presentation on a topic from the syllabus or related to the syllabus *Note: The student can submit only 1 assignment and give 1 PowerPoint presentations per semester. Respective college/department can decide the allocation* **FOR PAPER 3:** The students have to present a clinical case/trial study report.	30
2.	**a.** Active participation in routine class instructional deliveries	05
	b Overall conduct as a responsible student, w.r.t manners, skill in articulation, leadership qualities demonstrated through organizing co curricular activities, etc.	05
	Total Marks	40

Sr. No	Evaluation type	Marks
3.	**FOR PAPER 4:** The internal assessment will comprise of the following: a. Online course: The student is expected to complete at least one online course relevant for the subject from any of the appropriate reputed online platforms. A proof of successful completion of the online course must be provided for the award of marks.	20
	b. Research Proposal: The student is expected to submit a research proposal relevant to the subject.	20
	Total Marks	40

B. Theory -External examination -60%

Semester End Theory Assessment- 60 marks

- The duration of this exam will be of 2.5 hours (150 minutes)
- The theory question paper will have 5 questions each of 12 marks.

• For each unit, there will be one question and the fifth will be based on all the four units. • The fifth question will have 6 sub-questions out of which the student has to attempt any 3. • All questions shall be compulsory with internal choice within the questions such that each question will be set of 24 marks with options.

Practical Examination Evaluation scheme (50 marks per paper)

1.	Practical Question 1	25
2.	Practical Question 2	15
3.	Journal	5
4.	Viva Voce	5
	OR	
1.	Practical Question	40
2.	Journal	5
3.	Viva Voce	5
	Semester IV- Project Dissertation	100 Marks
	For semester IV it is mandatory for students to undergo Hands-on Project training in an established research laboratory or college laboratory for 4-6 months; This should involve one or more relevant instrumentation technique. Thesis on the same to be evaluated by the guide alternatively by an internal examiner for 50M based on the student's performance, written matter and experimentation. A certificate must be appended with the thesis. The external examiner will assess for 50M as a Presentation during practical exams. Marks allotted by Internal examiner would be scaled down if required as per university guidelines.	

A certified copy of the journal is essential to appear for the practical examination.

Note:

- In case of any changes in the above-mentioned evaluation scheme, the chairperson of examinations would provide the necessary details and the format.
- The practical examinations at a center would be evaluated by one external examiner assigned by the University and one internal examiner assigned by the college/department.

Department of Biotechnology

Sr. No.	Roll No.	Name of the student	Title of Project
1	13001	Kajol A. Khilare	Isolation, Characterization and Optimisation of Cellulase Producing Bacteria
2	13002	Siddhi S. Shinde	Formulation and Effect of Biofertilizers on Growth and Yield In Brinjal Under Greenhouse Conditions
3	13003	Prabodhini B. Kamble	Phytochemical Studies of Cicer Arietinum and Moringa Oleifera Plants as A Source of Biocoagulating Agent for Wastewater Treatment.
4	13004	Shivangi R. Tiwari	Screening, Characterization of Bacteria Isolated From Soil and Extraction, Optimization and Application of the Amylase Enzyme Produced by the Isolated Bacteria
5	13005	Afrin A. Shaikh	Comparative Analysis of Phytochemicals and in Vitro Biological Activities of Solvent Extracts of Piper Betle.
6	13006	Arun M. Nair	Optimization of Media for Growth of Salmonella Species and Antigen Extraction
7	13007	Sarath U. Nair	Nanoparticle Based Biofilter Using Carbonized Coconut Husk for Effluent Water Treatment
8	13008	Arya S. Vartak	Determination of Nutrient Content of Different Fresh and Dried Fruits and Vegetables.
9	13009	Prajwali P. Bhoir	Isolation of Pigment Producing Bacteria from Various Food Samples and Testing of Antimicrobial Activity of Bacterial Pigments.
10	13010	Mansi G. Goenka	Comparative Analysis of Phytochemical, Antioxidant, Antimicrobial Activity, Anti-Inflammatory Activity And Silver Nanoparticle Synthesis of Avecennia Alba And Avecennia Marina.
11	13011	Sakshi S. Tiwari	Study of the Effect of Trichoderma Enriched Pig Dung Compost In Enhancing Agricultural Productivity
12	13012	Prajapati Sonelalal.S.	Optimisation of Growth Parameters for Extraction of 'O' Antigen From Salmonella Paratyphi
13	13013	Yadav Sandeep R.	Comparative Analysis of Soil Nutrients Based on the Productivity Test on the Microbially and Chemically Treated Seeds.
14	13014	Gautam Kishankumar B.	Study The Effect of NPK Formulated Biofertilizer on The Chlorophyll Content of Plants.

12/7/23

Teacher Signature

Head of the Department

Head of the
Biotechnology Department
S. D. S. M. College,
Palghar (W) - 401 404

Sonopant Dandekar Shikshan Mandali's
SONOPANT DANDEKAR ARTS, V. S. APTE COMMERCE
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Code.: (02525) 252163, Prin : 252317 • Resi.: 252316
website : www.sdamcollege.com • Email:sdamcollege@yahoo.com
(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/23

CERTIFICATE

The work described in this project entitled "Comparative Analysis of Phytochemicals and In-vitro Biological Activities of Solvent extracts of Piper betle.

"has been carried out independently by Afrin Azad Shaikh Exam Seat no. 454031 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

10/07/23
Signature of Guide

Signature of H.O.D
Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/07/23 13/7/23
Signature of Examiner

Sonopant Dandekar Shikshan Mandali's
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/2023

CERTIFICATE

The work described in this project entitled "Optimization of Media for growth of Salmonella species and Antigen Extraction

"has been carried out independently by Arun Muralidharan Nair Exam Seat no. 4540328 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/7/23 13/09/23

Signature of Examiner

SonopantDandekarShikshanMandali's

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Website:www.Sdsmcollege.ComEmail:Sdsmcollege@Yahoo.Com

(NaacReaccredated: 'B' Grade)

Ref. No. : **Date:** 10/07/2023

CERTIFICATE

The work described in this project entiled **"DETERMINATION AND COMPARISON OF NUTREINT CONTENT OF DIFFERENT FRESH AND DRIED FRUITS AND VEGETABLE SAMPLE."** has been carried out **Ms.Vartak Arya Swapnil** independently by Exam seat no **4540335** with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide Signature of H.O.D Signature of Principal Signature of Examiner

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Sonopant Dandekar Shikshan Mandali's
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/2023

CERTIFICATE

The work described in this project entitled " Isolation, Characterization and Optimization of Cellulose Degrading bacteria. "has been carried out independently by Kajol Anil Khilare Exam Seat no. 4540327 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

10/07/23

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/07/'23 13/7/2023

Signature of Examiner

Sonopant Dandekar Shikshan Mandali's
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/2023

CERTIFICATE

The work described in this project entitled "Study the Effect of formulated Biofertilizer on the chlorophyll content of Spinacia oleracea and Trigonella foenum graecum."has been carried out independently by Kisharkumar Bindulal Gautam Exam Seat no. 4540324 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

10/07/23

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

10/07/23 15/7/23

Signature of Examiner

Sonopant Dandekar Shikshan Mandali's
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/23

CERTIFICATE

The work described in this project entitled "Comparative analysis of Phytochemical constituents, Antioxidant, Antimicrobial, Antiinflammatory, Silver nanoparticle synthesis & Anti-dyeing activity of Avecennia Alba & Avecennia Marina" has been carried out independently by Manu Gopal Goenka Exam Seat no. 4540325 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D
Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/07/23 13/7/23

Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 11/07/2023

CERTIFICATE

The work described in this project entitled "Phytochemical studies of cicer arietinum and moringa oleifera plants used as a source of Bio-coagulant Agent for water purification" has been carried out independently by Kamble Prabodhini Balaji Exam Seat no. 4540326 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

12/07/23

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/07/23 13/10/23

Signature of Examiner

Sonopant Dandekar Shikshan Mandali's
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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : _____

CERTIFICATE

The work described in this project entitled "Isolation, Characterization of Pigment producing bacteria From various Food samples And Testing of Antimicrobial Activity of Bacterial Pigments" has been carried out independently by Prajwali Pramod Bhoir Exam Seat no. 4540323 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

15/7/23 3/07/23

Signature of Examiner

Sonopant Dandekar Shikshan Mandali's

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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com

(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 12/7/23

CERTIFICATE

The work described in this project entitled "Study the effect of Pig dung & cow dung compost enriched with Trichoderma enhancing agricultural productivity of Brinjal & Sorghum seeds."has been carried out independently by Tiwari Sakshi Sunil Exam Seat no. 4540333 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

12/7/23

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

14/07/23 14/7/23

Signature of Examiner

Sonopant Dandekar Shikshan Mandali's
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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com
(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/2023

CERTIFICATE

The work described in this project entitled "Comparative analysis of Soil Based on the productivity of Biologically & chemically treated seeds of Moong, Brinjal, Jowar and Tomato" has been carried out independently by Sandeep Rammurat Yadav Exam Seat no. 4540336 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Aisa 10/07/'23
Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

14/7/23 Aisa 14/07/'23
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website : www.sdsmcollege.com • Email:sdsmcollege@yahoo.com
(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/7/2023

CERTIFICATE

The work described in this project entitled "Nanoparticle based membrane using carbonized coconut husk for Effluent water treatment

"has been carried out independently by Sarath Unnikrishnan Nair Exam Seat no. 4540329 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/7/23 18/07/23

Signature of Examiner

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/2023

CERTIFICATE

The work described in this project entitled "Screening, Characterization of Bacteria isolated from Soil & Extraction, optimization & Application of the Amylase Enzyme Produced by the isolated Bacteria" has been carried out independently by Shivangi Rajnarayan Tiwari Exam Seat no. 4540334 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide

Signature of H.O.D
Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

14/07/23 14/7/23

Signature of Examiner

SonopantDandekarShikshanMandali's

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Website:www.Sdsmcollege.ComEmail:Sdsmcollege@Yahoo.Com

(NaacReaccredated 'B' Grade)

Ref. No. : **Date .** 10-07-2023

CERTIFICATE

The work described in this project entitled "**formulation and effect of biofertilizers ongrowth and yield in plant brinjal *(solanum melongena)* under green house**" has been carried out **Ms. Shinde Siddhi Santosh** independently by Exam seat no **4540332** with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

10/07/23

Signature of Guide | Signature of H.O.D | Signature of Principal | Signature of Examiner

13/07/23

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

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The work described in this project entitled "Comparative Study of Growth Parameter on Salmonella Paratyphi A for optimum extraction of 'O' Antigen"has been carried out independently by Sonelal Santosh Kumar Prajapati Exam Seat no. 4540330 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

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Co-ordinator
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13/7/23 15/07/23

Signature of Examiner

DETERMINATION AND COMPARISON OF NUTREINT CONTENT OF DIFFERENT FRESH AND DRIED FRUITS AND VEGETABLE SAMPLE.

University of Mumbai

TOWARDS PARTIAL FULFILMENT OF THE DEGREE

OF

MASTERS OF SCIENCE IN BIOTECHNOLOGY

UNDER THE GUIDANCE OF

DR.SHILPA GHARAT

SUBMITTED BY

MS. ARYA SWAPNIL VARTAK

DEPARTMENT OF BIOTECHNOLOGY

SONOPANT DANDEKARSHIKSHAN MANDALI COLLEGE,

PALGHAR, 401404

M.Sc. BIOTECHNOLOGY (2022-23)

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(NaacReaccredated: 'B' Grade)

Ref. No. : **Date:** 10/07/2023

CERTIFICATE

The work described in this project entiled **"DETERMINATION AND COMPARISON OF NUTREINT CONTENT OF DIFFERENT FRESH AND DRIED FRUITS AND VEGETABLE SAMPLE."** has been carried out **Ms.Vartak Arya Swapnil** independently by Exam seat no **4540335** with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Signature of Guide Signature of H.O.D Signature of Principal Signature of Examiner

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आकृती केंद्र AKRUTI Centre

तारापूर महाराष्ट्र साइट - एनपीसीआयएल **Tarapur Maharashtra Site – NPCIL,**
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Ref: AKRUTI / 2022-23 / **Date: 01/06/2023**

CERTIFICATE

This is to certify that **Miss Arya Swapnil Vartak,** M.Sc. Biotechnology Student from Sonopant Dandekar Shikshan Mandli's M.H Mehta Science College, has completed his project work entitled, **"Determinations and Comparison of Nutrient Content of Different Fresh and Dried Fruits and Vegetables Sample"** in **AKRUTI Centre, TMS-NPCIL, Boisar** from 01/03/2023 to 31/05/2023.

During project work we have noticed that, she was found punctual, hardworking and inquisitive.

We wish the very best in all his future endeavors.

Best regards,

Dr. R. S. Pawar
(AKRUTI Coordinator)

AKRUTI CENTRE, BOISAR

DECLARATION

I, **Vartak Arya Swapnil**, Student of M.Sc. part 2 Biotechnology hereby declare that the project entitled **"DETERMINATION AND COMPARISON OF NUTRIENT CONTENT FROM DIFFERENT FRUITS AND VEGETABLES."**Submitted by me for the academic year 2022-23, is the actual work carried by me under the guidance of **Dr. ShilpaGharat** . I further state that this work is original and no part has been presented for any degree, Diploma or similar title of any University.

Date: 10/07/2023

Place: PALGHAR

Signature of the Guide,

Dr. ShilpaGharat

Assistant professor

Department of Biotechnology

ACKNOWLEDGMENT

I am highly indebted and express my deepest sense of gratitude to the Head of the department of Biotechnology, **Dr. ShilpaGharat** for her valuable guidance and suggestions throughout the research work and preparation of this project.

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Finally I wish to extend a warm thanks to everybody involved directly and indirectly with my work

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1. ABSTRACT:

Nutrition is the biochemical and physiological process by which an organism uses food to support its life. It provides organisms with nutrients, which can be metabolized to create energy and chemical structures. Failure to obtain sufficient nutrients can lead to malnutrition. This nutrients can be obtained for various fruits, vegetables, meat, eggs , etc. Not only fresh vegetables and fruits but also from the fruits and vegetables that are dried under sun or preserved using different chemicals in order through consume seasonal fruits or vegetables them throughout the year. Different nutrients like proteins, calcium, vitamins, carbohydrates etc. are obtained these fruits and vegetables.

In this study, we detect presence or absence of nutrients by using different methods. Also compare the nutrient that are present in fresh fruit and vegetable sample and dried fruit and vegetable powder. If the nutrients are absent in dried fruits and vegetable or if they are in lesser amount what effect it will have on body of individual consuming it.

Nutrient like protein and calcium were determined qualitatively. Protein was estimated by giving alkaline condition to both fresh as well as dried samples. Calcium was estimated by precipitation reaction in both fresh and dried samples. Carbohydrates were determined by colorimetric analysis phenol-sulfuric acid method. Antioxidant activity was colorimetricanalysis by DPPH method. Vitamin C was determined titrimetric analysis by DCPIP method. The concentration of nutrients were compared between fresh and dried fruits and vegetable sample. They were compared to know which samples are more suitable to be included in diet. Comparative studies suggested that dried samples contain less concentrations than fresh. The samples used were dried in foldable solar drier, AKRUTI center, BARC, Boisar.

(Keywords: nutrients, proteins, calcium, vitamins, carbohydrates, antioxidant activity, colorimeter, titration)

2. INTRODUCTION:

Fruits and vegetables are the important part of human diet. They are essential for intake of different nutrients. These nutrients are essential for growth and metabolism of human body. Intake of fruits and vegetables with good amount of nutrition is important to maintain health of every individual human being. Nutrients like protein, calcium, vitamins, carbohydrates (sugars), antioxidant activity, etc. in fruits and vegetables are important for health of human being.

Protein are macromolecules made up of polypeptides of amino acids. Proteins are the basis of many animal body structures (e.g. muscles, skin, and hair). Each molecule is composed of amino acids, which are characterized by presence of nitrogen and sometimes sulphur. The body requires amino acids to produce new proteins (protein retention) and to replace damaged proteins. Protein contains 16.8 kilojoules (4 kilocalories) per gram. The main source of proteins are fish, milk, meat, egg, etc. but it can be also obtained from different fruits and vegetables. Lack of protein can make you lose muscle mass, which in turn cuts your strength, makes it harder to keep body balanced, and slows down the metabolism. It can also lead to anaemia.

Calcium is a vital nutrient required in metabolism of human body. It is a common electrolyte, but also needed structurally (for muscle and digestive system health, bone strength, some forms neutralize acidity) may help clear toxins, provides signaling ions for nerve and membrane functions. The main source of calcium is green leafy vegetables. Calcium deficiency can reduce bone strength and lead to osteoporosis.

Vitamins are micronutrients essential for metabolism of human body. They play a role in maintaining a healthy diet and required for metabolism and biological processes. Among all vitamins, vitamin C is an essential micronutrient needed for normal metabolic function of the human body . Vitamin C, which also known as ascorbic acid, is a water-soluble and anti-oxidant compound that is generally obtained from fruits and vegetables, such as blueberry, orange, lemon, strawberry, pepper and tomato.Vitamin C is known to prevent cold disease, lower blood pressure and cholesterol levels . However, deficiency of Vitamin C is proved to lead to scurvy in humans.

Carbohydrates are most abundant class of organic compounds found in living organisms. Carbohydrates are a major source of metabolic energy, both for plants and animals. A diet that does not contain carbohydrates can lead to muscle breakdown, ketosis and dehydration. This can be prevented by 50 to 100gms of carbohydrate per day. Total sugars includes sugar naturally present in many nutritious foods and beverages such as in fruits and vegetables that may be present in product. They may occur naturally or be added to food product to provide nutrients and in most case to improve the textures and overall quality of the food product. Many polysaccharides are also added to the food system as a stabilizer and dietary fibre. For example locust bean and Guar gum are used to stabilize emulsion and prohibits ice crystals in ice creams.

Antioxidant plays essential roles in the maintaining of good health. Fruits and vegetables are the primary sources of antioxidants. Fruits and vegetables intake have shown a direct correlation

with healthy lifestyles, which may explain among the individual who adequately consumes fruits and vegetables every day. Higher fruits and vegetables intake are related to a healthy diet pattern correlated with lower CHD (chronic heart disease) incidence rates and negatively associated with the consumption of saturated fat-rich food. Fruits and vegetables are the main sources of antioxidants, which are the phytochemicals that prevent some of the processes of developing cancer and cardiovascular diseases. Antioxidants prevent the oxidation process of lipids or other molecules by hindering the initiation of oxidizing chain reactions where redox reactions are fundamental for the biological processes. Human body, at the time of ATP (adenosine triphosphate) production, cells use oxygen and release free radicals by the mitochondria. Where also Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are produced as a result of the cellular redox process. Although free radicals are considered as important factors for normal physiology, they cause cellular damage when produced in excess. Reactive Oxygen Species are associated with more than 100 diseases, like cancer and inflammatory conditions, atherosclerosis, hypertension, Alzheimer's disease, Parkinson's disease, ischemic diseases, etc.Antioxidants present in fruits and vegetables may help in decreasing the level of oxidative stress**[7].**

3. REVIEW OF LITERATURE:

Nazir Ahmed, Anwar Ali, SakhawatRiaz, Arslan Ahmad and Muhammad Aqib (2021)

Protein consumption of 0.8 grams per kilogram body weight per day (g/kg/d) [12], it is been shown that fit and healthy seniors need a protein intake of 1.2 g/kg/d to avoid age-related weight gain and function..

Deekshika B. , Praveena Lakshmi B, Hemant Singuluri and M.K. Sukumaran.(2015)

Estimation of ascorbic Content in fruits & vegetables from Hyderabad , India. The ascorbic acid content was estimated by titrimetric method. The results concluded that the fruits and vegetable available in market were rich in Vitamin C (ascorbic acid content) as should be included in diet as per availability and age of an individual.

Sheeba Ali Siddiqui and C.M.Noorjahan (2017)

Different micronutrients and macronutrients in vegetables were estimated by using different. It was found that the vegetables are rich in various nutrients such as proteins, calcium, fats, carbohydrates, vitamins and minerals.

Md.Jannatul Feradus, ZannatulFerdous, Rubyat Jahan Sara, Md. Omar Faruque (2020)

Estimation of total antioxidant activity of selected fruits and vegetables in Jashore region, Bangladesh . The estimation was done by DPPH method. The study concluded that selected fruits and vegetables were the potential sources of antioxidants and macromolecules.

MrinalKanti Si , Afshin Ali, Ankush Vaidya , Sudhakar Singh, KajalDeshmukh , Neha Bhagat, PunamHanwat, NandiniSahu, PallaviBhagat, SakshiTilase, ShrutiShriwastri. (2021)

Determination of calcium, magnesium, iron was done by titrimetric method. The study revealed that fruits and vegetables are rich in calcium, magnesium and iron. Thus, it should be included in diet of a person having iron and calcium deficiency.

4. RATIONALE:

Background: Nutrients are essential for growth development and metabolism of human beings. Proper intake ofnutrients are essential through balanced diet. This nutrients can be obtained from different fruits and vegetables. Preserved or dried fruits and vegetables have been also a part of diet. The nutrition content of food can be determined by various methods using both qualitative and quantitative method. Nutrients like proteins, calcium, antioxidant activity, carbohydrates and vitamins can be estimated.

Improper diet leads to lack of nutrition. Lack of nutrition can lead to various diseases and disorder or ill condition. Also improper nutrition in children can lead to malnutrition. Therefore, in order to avoid diseases and lead a healthy life balanced diet is necessary.

The project topic is DETERMINATION AND COMPARISON OF NUTREINT CONTENT OF DIFFERENT FRESH AND DRIED FRUITS AND VEGETABLE SAMPLES.

In foldable solar dryer (AKRUTI centre, BARC,Boisar) we dry fresh fruits & vegetable samples and the test them for their nutrient content using instruments like colorimeter and titration method.

Using different fruit and vegetable samples, both fresh and dried. This samples help to compare the nutrient content of fruits and vegetables in its fresh form and its content when it is dried, preserved and consumed.

5. AIM AND OBJECTIVE:

AIM:

Determination and compare of nutritional content of fruits and vegetables (fresh and dried) collected from market of Palghar and Boisar.

OBJECTIVE:

1) To determine presence or absence of proteins in fresh and dried fruits and vegetable samples.
2) To determine presence or absence of calcium in fresh and dried fruits and vegetable samples.
3) To estimate carbohydrates present in fresh and dried samples usingfresh and dried fruits and vegetable samples by Phenol-Sulfuric Acid method.
4) To determine antioxidant activity of fresh and dried fruits and vegetable samples using DPPH method.
5) To determine Vitamin C content in fresh and dried fruits and vegetables samples using DCPIP.
6) To compare the nutrient contents between fresh and dried samples.

6. MATERIALSAND METHODS:

Different fruit and vegetables samples were collected from the market of Palghar and Boisar region.

Fruit samples like:

1) *Manilkarazapota* (Chikoo)
2) *Mangiferaindica*(Raw mango)
3) *Psidiumguajava*(Guava)
4) *Ananascomosus*(Pineapple)
5) *Solanumlycopersicum*(Tomato)
6) *Cucurbita moschata*(Pumpkin)
7) *Citrus limon*(Lemon)
8) *Phyllanthusemblica*(Gooseberry)

Vegetable samples like:

1) *Trigonellafoenum-graecum*(Fenugreek)
2) *Zingiberofficinale*(Ginger)
3) *Capsicum annuum*(Green chilli)
4) *Beta vulgaris L.*(Beetroot)
5) *Ipomoea batatas*(Sweet potato)
6) *Spinaciaoleracea L.*(Spinach)
7) *Coriandrumsativum*(Coriander)
8) *Lagenariasiceraria*(Bottle gourd)
9) *Murrayakoenigii*(Curry leaves)
10) *Amaranthusviridis*(Green Amaranthus)
11) *Amaranthuscruentus*(Red Amaranthus)
12) *Momordicacharantia*(Bitter gourd)
13) *Solanummelongena*(Brinjal)
14) *Daucuscarota subsp. Sativus*(Carrot)
15) *Allium cepa*(Onion)
16) *Brassica oleracea*(Broccoli)
17) *Cocciniagrandis*(Ivy gourd)
18) *Agaricusbisporus*(Mushroom)
19) *Amorphophalluspaeoniifolius*(Elephant foot yam)
20) *Phaseolus vulgaris*(Green beans)
21) *Mentha*(Mint)
22) *Leptospermum petersonii*(Lemon tea leaves)

Flowers like:

1) *Tagetes*(Marigold)

The above samples were dried in foldable solar drier (AKRUTI Centre, BARC, Boisar).The dried samples were grinded using electric grinder into fine powder.

Figure 1:Foldable solar dryer,AKRUTI centre, BARC, Boisar.

6.1 Preparation of samples:

- The fresh samples were washed. For preparation of extract the samples were crushed using mortar pestle and distilled water was used as a solvent.
- The suspension of dried samples was prepared using distilled water. To reduce turbidity the suspension was filtered using filter paper.

Figure 2: Mortar and Pestle (image credit:utest.in)

6.2 Qualitative test of proteins

When proteins and peptides (i.e. peptide bonds) treated with an alkaline solution of dilute copper sulfate a violet color is formed. A positive test is indicated by the formation of a violet color.

Requirements:

Samples	1) Fresh sample extract 2) Dried sample suspension
Chemicals	1) 5% NaOH 2) 2% $CuSO_4$
Glasswares	1) Test-tube 2) Pipettes 3) Beaker 4) Dropper
Miscellaneous	Test-tube stand.

Table 1: Requirement table fordetermination of protein.

Procedure:

1) 5 ml of NaOH was added to the test tube.
2) 1ml of both fresh and dried samples extract and suspension were added to the test tube.
3) Then to which 2-3 drops of $CuSO_4$ was added. After addition for development of colour the tubes were incubated at room temperature for 5-10min. Bluish – violet colour indicated presence of protein in the samples. The intensity of colour helps to determine the amount of protein that might be present in the sample.

6.3Qualitative testing of calcium.

It is a Precipitation reaction to remove cations sequentially from a mixture.

Requirements:

Sample	1) Fresh sample extract 2) Dried sample suspension
Chemicals	1) 1% Ammonium chloride 2) 1% Ammonium oxalate
Glasswares	1) Test-tube 2) Pipettes 3) Funnel 4) Beaker
Miscellaneous	Filter paper Test tube stand

Table2: Requirement table for determination of calcium.

Procedure:

1. 1ml of both fresh and dried samples were added to the tube .
2. Add 1 ml of ammonium chloride . The tubes were incubated at room temperature for 2minutes.
3. Then the solution in the tube was filtered using filter paper.
4. To the filtrate1 ml of ammonium oxalate was added which developed white precipitate if the calcium was present in the sample.

6.4 Estimation of carbohydrate by Phenol-Sulfuric Acid Method

Estimation of total sugar present in different fruits sample can be determined colorimetrically by Phenol-Sulfuric Acid Method. The method detects all classes of carbohydrates including mono-, di, oligo- and polysaccharides. In hot and strong acidic medium glucose is dehydrated to hydroxy methyl furfural, this forms a yellow brown coloured product with phenol, that can be measured colorimetrically at 480 nm. The sulphuric acid causes all non reducing sugar to be converted to reducing sugar so that this method determines the total sugar present in foods.

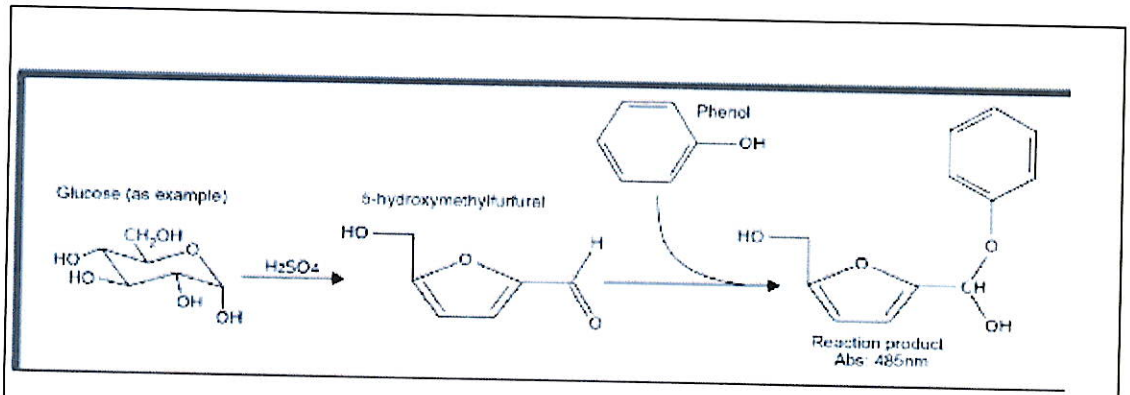

Figure 3: Reaction of total sugar.

Requirements:

Sample	1) Fresh sample extract 2) Dried sample suspension
Chemicals	1) 1mg/ml standard glucose 2) 5% phenol 3) Concentrated sulfuric acid
Glasswares	1) Test-tube 2) Pipettes 3) Beaker
Instrument	1) Incubator set at 37°C 2) Colorimeter set at 480nm
Miscellaneous	1) Test-tube stand 2) Tissue paper

Table 3: Requirement table for total sugar estimation.

Protocol:

1. Take 8 test tube, label them as blank, unknown and as per dilution
2. Make dilution of glucose std solution (200 µg/ml) using distilled water as diluent that is 40, 80,120, 160 and 200 Adjusting it to a total volume 1.
3. Add 1 ml of 5% Phenol solution to all the tubes.
4. Add 5 ml of Conc. Sulfuric acid to each tube and mix it well.
5. Stand all the tubes for 10 min, mix the contents of all the tubes and place in an incubator set at 37°C for 15 min.
6. Switch on the Colorimeter, select the wavelength of 490 nm.
7. First take the absorbance of blank and make it zero.
8. After that take OD of all the tube.Wash the cuvette each time after taking OD.
9. Plot a standard graph of absorbance at 480nm on Y-axis versus Conc. Of glucose in mg/ml on X-axis.

Dilution table:

Dilution scheme:

Stock used: 200µg/ml Std. Glucose
Diluent used: Distilled water

Tube no.	Glucose Conc. (µg/ml)	Amount of stock (ml)	Amount of diluent (ml)	Total volume (ml)	5% phenol (ml)	Conc. Sulphuric acid (ml)		OD at 480nm
1	Blank	-	1.0	1.0	1.0	5.0		
2	40	0.2	0.8	1.0	1.0	5.0	Mix and stand for 10 min. After that incubate at 37^0Cfor 20 min.	
3	80	0.4	0.6	1.0	1.0	5.0		
4	120	0.6	0.4	1.0	1.0	5.0		
5	160	0.8	0.2	1.0	1.0	5.0		
6	200	1.0	-	1.0	1.0	5.0		
7	Fresh vegetable and fruit sample (1:10 diluted)	-	-	1.0	1.0	5.0		
8	Dried vegetable and fruit sample (1:10 diluted)	-	-	1.0	1.0	5.0		

Table 4: Dilution table for total sugars.

6.5 Determination of antioxidant activity by DPPH method

Free radicals are inevitably produced in biological systems and also encountered exogenously, and are known to cause .Various degenerative disorders like mutagenesis, carcinogenesis, cardiovascular disturbances an ageing. Antioxidants are the compound which combat the free radicals by intervening at any one of the three major step of the free radical mediated oxidative process, viz. initiation, propagation and termination. These antioxidants are also produced by biological system and occurs naturally in many microorganisms, algae, plants etc.

To determine the antioxidant activity of extracts a stable Alpha diphenyl beta picrylhydrazyl (DPPH; M=394.33) is used. The assay is based on the measurement of the scavenging activity of antioxidants towards it. The odd electron of nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine. DPPH is characterized as a stable free radical by the delocalization of the spare electron over the molecule as a whole so that the molecules do not dimerize like most other free radical. The delocalization also give rise to the deep violet colour with an absorption in methanol solution at around 520nm. On mixing DPPH solution with a substance that can donate a hydrogen atom it give rise to the reduced form with the loss of violet colour representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is where ZH is the reduced form and A• is the free radical produced in the first step.

Requirements:

Sample	1)Fresh sample extract 2) Dried sample suspension
Reagents	1) 0.01mM of DPPH (4mg in absolute methanol) Store it in brown bottle 2)Absolute ethanol 3) Std. Ascorbic acid (mg/ml)
Glasswares	1)Test-tube 2) Pipette 3)cuvette
Instruments	1)Colorimeter set at 520nm 2)Weighing machine
Miscellaneous	1)Tissue paper 2)Test-tube stand

Table 5:Requirement table for determination of antioxidant activity.

Protocol:

1) Weigh 1gm of sample (both fresh and dried fruit and vegetables).
2) Crush the fresh samples in mortar pestle using 5ml of ethanol.
3) Filter the solution using muslin cloth.
4) Use the filtrate as sample.

Protocol table:

Concentration of standard (mg/ml)	Volume of standard (ml)	Volume of methanol (ml)	Total volume (ml)	Amount of DPPH (ml)
Blank	-	5.0	5	3.0
0.2	1.0	4.0	5	3.0
0.4	2.0	3.0	5	3.0
0.6	3.0	2.0	5	3.0
0.8	4.0	1.0	5	3.0
1.0	5.0	-	5	3.0
Control	-	-	-	3.0
Unknown (samples)	1.0(samples)	-	1ml	3.0

Incubate in dark condition for 30 min and take O.D. at 520nm (Table 6: Dilution table for antioxidant activity.)

6.6 Determination of concentration of Vitamin C by DCPIP method.

Titration method is used to determine concentration of Vitamin C different food samples . A known concentration of titrant is allowed to react with an analyte of unknown concentration. Using calibrated burette it is possible to determine the exact amount of titrant that has been consumed when the end point is reached, which is determined by the colour change of the indicator.

Ascorbic acid is reducing agent whose amount in the sample can be determined by redox titration using reaction between Ascorbic acid and 2,6-dichlorophenolindophenol (2,6-DCPIP). DCPIP is used as titrant it oxidizes ascorbic acid and acts as a self indicator. To be a self indicator, a substance must be of one colour in presence of excess analyte, and of another colour when the analyte has all reacted .

Theascorbic acid content of a sampledichlorophenolindophe can be determined by redox titration method. The dichlorophenolindophenol (DCPIP) (a blue solution in oxidized state) solution is reduced by ascorbic acid to a colourless solution ($DCPIPH_2$). During this process, ascorbic acid in turn is oxidized to dehydro-ascorbic acid, which in acidic medium gives light pink colour.

REACTION:

1) $C_6H_8O_6 \rightarrow C_6H_6O_6 + 2e^- + 2H^+$
2) DCPIP (blue) + $H^+ \rightarrow$ DCPIPH (pink)
3) DCPIPH (pink) + vit. C $\rightarrow$ $DCPIPH_2$

In this titration, when all ascorbic acid in the solution is used up, there are no electrons available to reduce DCPIPH further and hence the solution remains pink (DCPIPH). The end point is a pink colour that persists for 30 seconds or more, as there is not enough ascorbic acid to reduce all of the DCPIPH.

$$C_6H_6O_6 + C_{12}H_7C_{l2}NO_2 \rightarrow \quad C_6H_6O_6 + C_{12}H_9Cl_2NO_2$$

The amount of 2,6-DCPIP required for known amount of standard ascorbic acid is estimated first and this is used as a reference for estimation of ascorbic acid in the sample. Oxalic acid is added to prevent oxidation of ascorbic acid due to molecular oxygen in air.

Requirements:

Sample	1)Fresh sample extract 2) Dried sample suspension
Reagents	1. 4% oxalic acid 2. 2,6-dichlorophenolindophenol 3. 1mg/ml Ascorbic acid (stock solution) 4. 100ug/ml Ascorbic acid (working solution)
Glasswares	1. Conical flasks 2. 10ml pipette 3. Burette 4. Beaker
Miscellaneous	1. Burette stand 2. Mortar and pestle 3. Measuring cylinder 4. Filter paper

Table 7: Requirement table for determination of ascorbic acid.

Procedure:

A) Determination of ascorbic acid content.

a) For standard:

1) Take 5ml of ascorbic acid working standard in the conical flask.
2) Add 10ml of 4% oxalic acid.
3) The contents in the flask were titrated against the dye solution unit the appearance of a pale pink colour that persisted for a few min
4) Note the titration reading (V_1)

b) For Test sample:

1) Take 5ml of sample in the conical flask.
2) Add 10ml of 4% oxalic acid.
3) The contents in the flask were titrated against the dye solution until the appearance of a pale pink colour that persisted for a few min.
4) Note the titration reading (V_2).

7. INSTRUMENTS USED:

7.1. Solar drier

Figure 4: Foldable solar dryer AKRUTI centre, BARC, Boisar

Foldable solar dryer is a rectangular box with triangular top. The solar radiations are absorbed by black mat metallic outer surface of dryer. Heat is absorbed by inside air through conducting metallic plates. The geometry of the solar dryer ensures that the resultant hot air moves up and heats the product evenly and dries it by evaporating trapped moisture. Finally, this air escapes through vent at top. The dryer is of modular type and can be easily dismantled into a thin rectangular box for easy transportation & storage.

Applications

The foldable solar dryer in the capacity of 10, 25 & 100 kg is used for the preparation of raisin, drying of grapes, onion, mango pulp, green leafy vegetables, Gooseberry (Amla), jack fruit pulp, ginger, green pepper, herbal medicines etc.

7.2. Colorimeter

Figure 5: Colorimeter and cuvettes (img credit: indiamart)

Colorimeter is a light-sensitive device that helps certain solutions absorb a particularwavelength of light in colorimetry. It is used to measure the absorbance and transmittance of light that passes through a liquid. Colorimeter also determines concentration of a coloured Compound in a solution. Principle of Colorimeter is that coloured compounds can absorb a certain wavelength of light when monochromatic light is passed through them. The working of a colorimeter is based on the concept of Beer-Lambert's law. (College duniya.com).

8. OBSERVATION:

8.1. For qualitative estimation of proteins:

A) For fruits:

Sample used	Colour developed	Presence or absence of proteins
1) Fresh*Manilkarazapota* (Chikoo)	Bluish-violet	Presence of protein
2) Dried*Manilkarazapota* (Chikoo)	Brown	Absence of protein
3) Fresh*Mangiferaindica*(Raw mango)	Bluish-violet	Presence of protein
4) Dried*Mangiferaindica*(Raw mango)	Brown	Absence of protein
5) Fresh*Psidiumguajava*(Guava)	Orange colour	Absence of protein
6) Dried*Psidiumguajava*(Guava)	White colour	Absence of protein
7) Fresh*Ananascomosus* (Pineapple)	Pale blue	Presence of protein
8) Dried*Ananascomosus* (Pineapple)	Orange colour	Absence of protein
9) Fresh*Solanumlycopersicum*(Tomato)	Bluish-violet	Presence of protein
10) Dried*Solanumlycopersicum*(Tomato)	Bluish-green	Presence of protein
11) Fresh*Cucurbitamoschata*(Pumpkin)	Orange colour	Absence of protein
12) Dried*Cucurbitamoschata*(Pumpkin)	Orange colour	Absence of protein
13) Fresh *Citruslimon*(Lemon)	Blue colour	Presence of protein

14) Dried *Citrus limon*(Lemon)	Bluish-green	Presence of protein
15) Fresh *Phyllanthusemblica* (Gooseberry)	Orange colour	Absence of protein
16) Dried*Phyllanthuse mblica* *(*Gooseberry)	Orange colour	Absence of protein

Table 8- Observation of protein test for fruits.

B) For vegetables

Sample used	Colour developed	Presence or absence of proteins
1) Fresh *Trigonellafoenum-graecum*(Fenugreek)	Green colour	Absence of protein
2) Dried*Trigonellafoenum-graecum*(Fenugreek)	Green colour	Absence of protein
3) Fresh *Capsicum annuum*(Green chilli)	Blue colour	Presence of protein
4) Dried*Capsicum annuum*(Green chilli)	Brown colour	Absence of protein
5) Fresh*Zingiberofficinale* (Ginger)	Green colour	Absence of protein
6) Dried*Zingiberofficinale* (Ginger)	Brown colour	Absence of protein
7) Fresh *Beta vulgaris L.*(Beetroot)	Blue colour	Presence of protein
8) Dried*Beta vulgaris L.*(Beetroot)	Brown colour	Absence of protein
9) Fresh *Ipomoea batatas*(Sweet potato)	Bluish-violet	Presence of protein
10) Dried *Ipomoea batatas*(Sweet potato)	Bluish-violet	Presence of protein
11) Fresh *Spinaciaoleracea L.* (Spinach)	Bluish green	Presence of protein

12) Dried *Spinaciaoleracea L.*(Spinach)	Brown colour	Absence of protein
13) Fresh*Coriandrumsativum*(Coriander)	Green colour	Absence of protein
14) Dried*Coriandrumsativum*(Coriander)	Brown colour	Absence of protein
15) Fresh*Lagenariasiceraria*(Bottle gourd)	Blue colour	Presence of protein
16) Dried*Lagenariasiceraria*(Bottle gourd)	Orange colour	Absence of protein
17) Fresh*Murrayakoenigii*(Curry leaves)	Green colour	Absence of protein
18) Dried*Murrayakoenigii*(Curry leaves)	Green colour	Absence of protein
19) Fresh *Amaranthusviridis*(Green Amaranthus)	Green colour	Absence of protein
20) Dried*Amaranthusviridis*(Green Amaranthus)	Green colour	Absence of protein
21) Fresh*Amaranthuscruentus*(Red Amaranthus)	Green colour	Absence of protein
22) *Dried Amaranthuscruentus*(Red Amaranthus)	Brown colour	Absence of protein
23) Fresh*Momordicacharantia*(Bitter gourd)	Bluish-violet	Presence of protein
24) Dried*Momordicacharantia*(Bitter gourd)	Bluish-violet	Presence of protein

25) Fresh*Solanummelongena*(Brinjal)	White colour	Absence of protein
26) Dried*Solanummelongena*(Brinjal)	White colour	Absence of protein
27) Fresh*Daucuscarota subsp. Sativus*(Carrot)	Bluish-violet	Presence of protein
28) Dried *Daucuscarota subsp. Sativus*(Carrot)	Pale blue	Presence of protein
29) Fresh *Allium cepa*(Onion)	Bluish-violet	Presence of protein
30) Dried *Allium cepa*(Onion)	Yellow colour	Absence of protein
31) Fresh *Brassica oleracea*(Broccoli)	Bluish-violet	Presence of protein
32) Dried*Brassica oleracea*(Broccoli)	Bluish-violet	Presence of protein
33) Fresh*Cocciniagrandis*(Ivy gourd)	Blue colour	Presence of protein
34) Dried*Cocciniagrandis*(Ivy gourd)	Pale yellow	Absence of protein
35) Fresh *Agaricusbisporus*(Mushroom)	Bluish-violet	Presence of protein
36) Dried *Agaricusbisporus*(Mushroom)	Pale blue	Presence of protein

37) Fresh *Amorphophalluspaeoniifolius* (Elephant foot yam)	Bluish-violet	Presence of protein
38) Dried *Amorphophalluspaeoniifolius* (Elephant foot yam)	Blue colour	Presence of protein
39) Fresh*Phaseolus vulgaris*(Green beans)	Blue colour	Presence of protein
40) Dried*Phaseolus vulgaris*(Green beans)	Yellow colour	Absence of protein
41) Fresh*Mentha*(Mint)	Green colour	Absence of protein
42) D ried*Mentha*(Mint)	Brownish-black	Absence of protein
43) Fresh*Leptospermum petersonii*(Lemon tea leaves)	Green colour	Absence of protein
44) Dried*Leptospermum petersonii*(Lemon tea leaves)	Green colour	Absence of protein

Table 9: Observationsfor protein test for vegetables.

C) For flower:

Sample used	Colour developed	Presence or absence of proteins
1) Fresh*Tagetes*(Marigold)	Blue colour	Presence of protein
2) Dried *Tagetes (*Marigold)	Green colour	Absence of protein

Table 10: Observations for protein test for flower.

Observation for protein:

Figure 6 & 7: Observation for protein test of dried fruit and vegetables

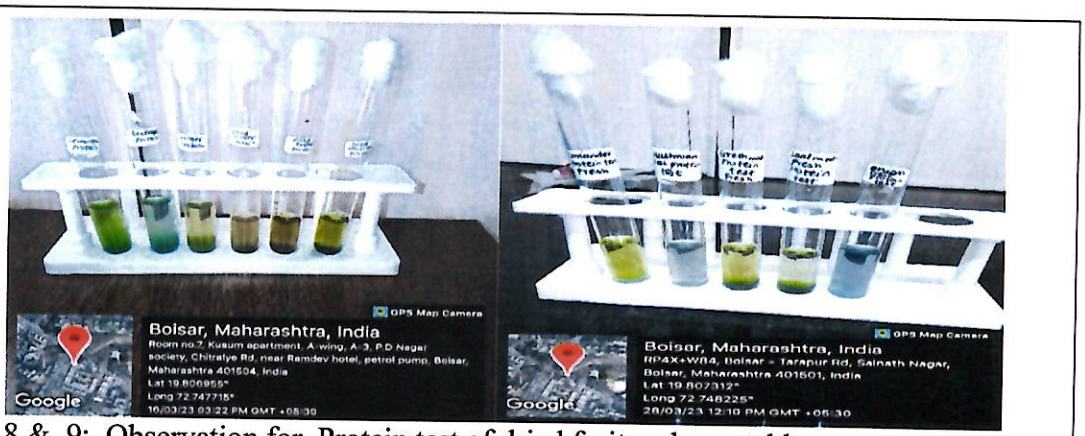

Figure 8 & 9: Observation for Protein test of dried fruit and vegetables

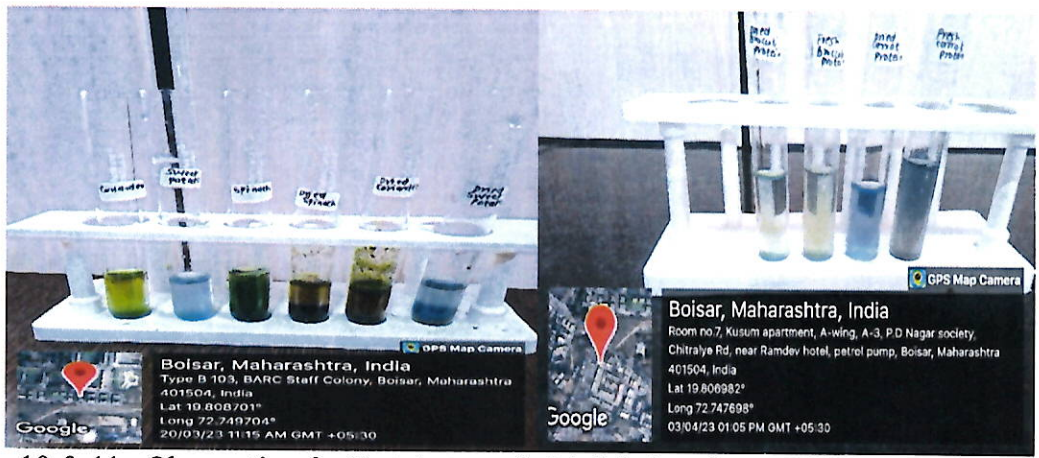

Figure 10 & 11: Observation for Protein test of dried fruit and vegetables.

Figure 12 & 13 : Observation for protein test of dried fruit and vegetables.

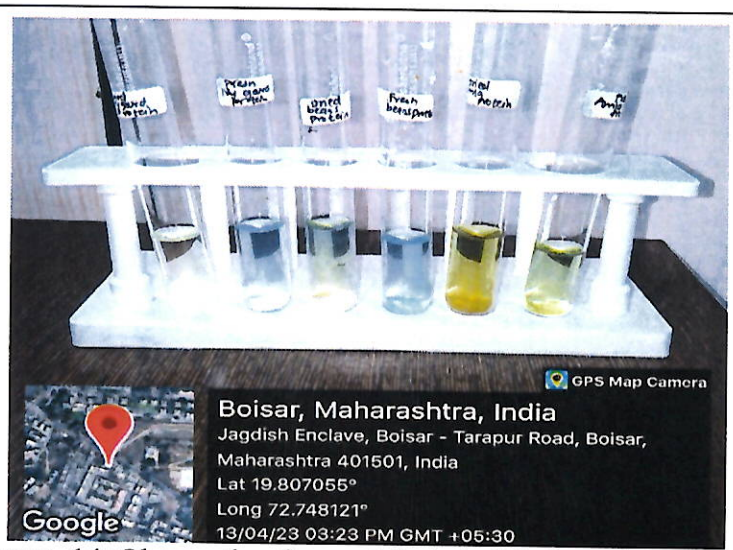

Figure 14: Observation for protein test of fruits and vegetables.

Figure 15 : Observation for protein test of fruits and vegetables.

8.2 For qualitative test of calcium:

A) For fruits:

Samples used	Formation of white precipitate	Presence or absence of calcium
1) Fresh*Manilkarazapota* (Chikoo)	White ppt.	Presence of calcium
2) Dried *Manilkarazapota* (Chikoo)	White ppt.	Presence of calcium
3) Fresh *Mangiferaindica*(Raw mango)	White ppt.	Presence of calcium
4) Dried *Mangiferaindica*(Raw mango)	White ppt.	Presence of calcium
5) Fresh*Psidiumguajava*(Guava)	White ppt.	Presence of calcium
6) Dried *Psidiumguajava*(Guava)	White ppt.	Presence of calcium
7) Fresh*Ananascomosus* (Pineapple)	White ppt.	Presence of calcium
8) Dried *Ananascomosus* (Pineapple)	White ppt.	Presence of calcium
9) Fresh*Solanumlycopersicum*(Tomato)	White ppt.	Presence of calcium
10) Dried *Solanumlycopersicum*(Tomato)	Yellow colour was observed	Absence of calcium
11) Fresh*Cucurbita moschata*(Pumpkin)	White ppt.	Presence of calcium
12) Dried *Cucurbita moschata*(Pumpkin)	White ppt.	Presence of calcium
13) Fresh *Citrus limon*(Lemon)	White ppt.	Presence of calcium
14) Dried *Citrus limon*(Lemon)	Yellow colour was observed	Absence of calcium
15) Fresh *Phyllanthusemblica*(Goose	White ppt.	Presence of calcium

berry)		
16) Dried *Phyllanthusemblica*(Goose berry)	White ppt.	Presence of calcium

Table 11: Observations for calcium test of fruits.

B) For vegetables:

Samples used	Formation of white precipitate	Presence or absence of calcium
1) Fresh *Trigonellafoenum-graecum*(Fenugreek)	Green colour was observed	Absence of calcium
2) Dried *Trigonellafoenum-graecum*(Fenugreek)	Yellow colour was observed	Absence of calcium
3) Fresh*Capsicum annuum*(Green chilli)	White ppt.	Presence of calcium
4) Dried *Capsicum annuum*(Green chilli)	White ppt.	Presence of calcium
5) Fresh*Zingiberofficinale* (Ginger)	White ppt.	Presence of calcium
6) Dried*Zingiberofficinale* (Ginger)	White ppt.	Presence of calcium
7) Fresh *Beta vulgaris L.*(Beetroot)	Red colour was observed	Absence of calcium
8) Dried *Beta vulgaris L.*(Beetroot)	Red colour was observed	Absence of calcium
9) Fresh *Ipomoea batatas*(Sweet potato)	White ppt.	Presence of calcium
10) Dried *Ipomoea batatas*(Sweet potato)	White ppt.	Presence of calcium
11) Fresh *Spinaciaoleracea L. (*Spinach)	Brown colour was observed	Absence of calcium
12) Dried *Spinaciaoleracea L. (*Spinach)	Green colour was observed	Absence of calcium
13) Fresh*Coriandrumsativum*(Coriander)	Brown colour was observed	Absence of calcium
14) Dried *Coriandrumsativum*(Coriander)	Brown colour was observed	Absence of calcium
15) Fresh*Lagenariasiceraria*(Bottle gourd)	White ppt.	Presence of calcium
16) Dried *Lagenariasiceraria*(Bottle gourd)	White ppt.	Presence of calcium
17) Fresh*Murrayakoenigii*(Curry leaves)	White ppt.	Presence of calcium

18) *Dried Murrayakoenigii*(Curry leaves)	Brown colour	Absence of calcium
19) Fresh *Amaranthusviridis*(Green Amaranthus)	White ppt.	Presence of calcium
20) Dried *Amaranthusviridis*(Green Amaranthus)	White ppt.	Presence of calcium
21) Fresh*Amaranthuscruentus*(Red Amaranthus)	Red colour was observed	Absence of calcium
22) Dried *Amaranthuscruentus*(Red Amaranthus)	Red colour was observed	Absence of calcium
23) Fresh *Momordicacha* 24) *rantia*(Bitter gourd)	White ppt.	Presence of calcium
25) Dried*Momordicacharantia*(Bitter gourd)	White ppt.	Presence of calcium
26) Fresh*Solanummelongena*(Brinjal)	Brown colour wasobserved	Absence of calcium
27) Dried *Solanummelongena*(Brinjal)	Brown colour wasobserved	Absence of calcium
28) Fresh*Daucuscarota subsp. Sativus*(Carrot)	White ppt.	Presence of calcium
29) Dried *Daucuscarota subsp. Sativus*(Carrot)	White ppt.	Presence of calcium
30) Fresh *Allium cepa*(Onion)	White ppt.	Presence of calcium
31) Dried *Allium cepa*(Onion)	White ppt.	Presence of calcium
32) Fresh *Brassica oleracea*(Broccoli)	White ppt.	Presence of calcium
33) Dried *Brassica oleracea*(Broccoli)	Green colour was observed	Absence of calcium
34) Fresh*Cocciniagrandis*(Ivy gourd)	White ppt.	Presence of calcium
35) Dried *Cocciniagrandis*(Ivy gourd)	White ppt.	Presence of calcium
36) Fresh *Agaricusbisporus*(Mushroom)	White ppt.	Presence of calcium
37) Dried *Agaricusbisporus*(Mushroom)	White ppt.	Presence of calcium

38) Fresh *Amorphophalluspaeoniifolius* (Elephant foot yam)	White ppt.	Presence of calcium
39) Dried *Amorphophalluspaeoniifolius* (Elephant foot yam)	White ppt.	Presence of calcium
40) Fresh *Phaseolus vulgaris*(Green beans)	White ppt.	Presence of calcium
41) Dried *Phaseolus vulgaris*(Green beans)	White ppt.	Presence of calcium
42) Fresh *Mentha*(Mint)	Brown colour was observed	Absence of calcium
43) Dried *Mentha*(Mint)	Brown colour was observed	Absence of calcium
44) Fresh *Leptospermum petersonii*(Lemon tea leaves)	White ppt.	Presence of calcium
45) Dried *Leptospermum petersonii*(Lemon tea leaves)	Yellow colour was observed	Absence of calcium

Table 12: Observations for calcium test of vegetables.

C) For flower:

Sample used	Formartion of white precipitate	Presence or absence of calcium
1) Fresh*Tagetes* (Marigold)	Orange colour was observed	Absence of calcium
2) Dried *Tagetes* (Marigold)	Orange colour was observed	Absence of calcium

Table 13: Observation for calcium test of flower.

Observation for Calcium:

.Figure 16 &17: Observation for calcium test of fruits & vegetables.

Figure 18 & 19: Observation for calcium test of fruits & vegetables.

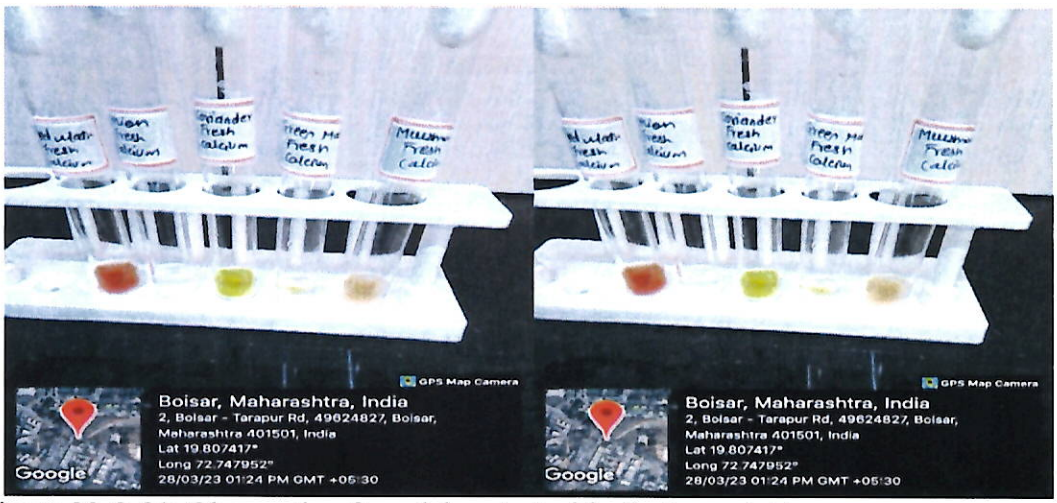

Figure 20 & 21: Observation for calcium test of fruits & vegetables.

Figure 22 & 23: Observation for calcium test of fruits & vegetables

8.3.Observation for determination of carbohydrates (total sugar)

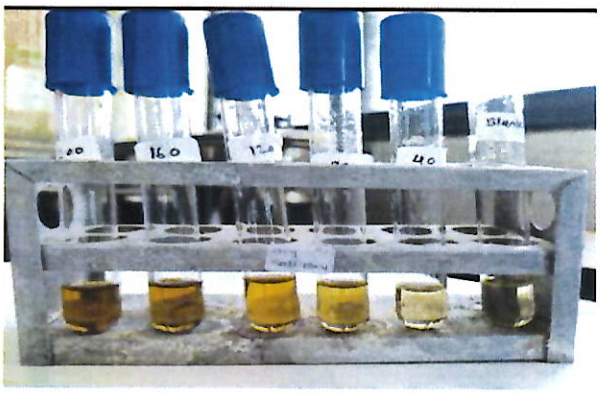

Figure 24: Standard of total sugars

- Samples used were 1:10 diluted

Tube no,	Conc. Of glucose (µg/ml)	Amount of stock (ml)	Amount of diluents (ml)	Total volume (ml)	5% Phenol (ml)	Conc. Sulfuric acid (ml)		O.D. at 480nm
1.	Blank	–	1.0	1.0	0.2	1.0	Mix & incubate at R.T. for 10 min. & then at 37^{0}C for 20 min.	0.00
2.	40	0.2	0.8	1.0	0.2	1.0		0.12
3.	80	0.4	0.6	1.0	0.2	1.0		0.16
4.	120	0.6	0.4	1.0	0.2	1.0		0.24
5.	160	0.8	0.2	1.0	0.2	1.0		0.50
6.	200	1.0	–	1.0	0.2	1.0		0.56
7.	Fresh Chikoo			1.0	0.2	1.0		0.35
8.	Dried Chikoo				0.2	1.0		0.17
9.	Fresh Raw mango				0.2	1.0		0.56
10.	Dried Raw				0.2	1.0		0.54

	mango							
11.	Fresh Guava				0.2	1.0		0.54
12.	Dried Guava				0.2	1.0		0.52
13.	Fresh Pineapple				0.2	1.0		0.54
14.	Dried Pineapple				0.2	1.0		0.51
15.	Fresh Tomato				0.2	1.0		0.30
16.	Dried Tomato				0.2	1.0		0.10
17.	Fresh Pumpkin				0.2	1.0		0.40
18.	Dried Pumpkin				0.2	1.0		0.38
19.	Fresh Lemon				0.2	1.0		0.26
20.	Dried Lemon				0.2	1.0		0.16
21.	Fresh Gooseberry				0.2	1.0		0.28
22.	Dried Gooseberry				0.2	1.0		0.24
23.	Fresh Fenugreek				0.2	1.0		0.26
24.	Dried Fenugreek				0.2	1.0		0.22
25.	Fresh Green chilli				0.2	1.0		0.56
26.	Dried Green chilli				0.2	1.0		0.44
27.	Fresh Ginger				0.2	1.0		0.30
28.	Dried Ginger				0.2	1.0		0.28
29.	Fresh Beetroot				0.2	1.0		0.50
30.	Dried Beetroot				0.2	1.0		0.49
31.	Fresh Sweet potato				0.2	1.0		0.50
32.	Dried Sweet potato				0.2	1.0		0.26
33.	Fresh spinach				0.2	1.0		0.15
34.	Dried spinach				0.2	1.0		0.06
35.	Fresh Coriander				0.2	1.0		0.31
36.	Dried Coriander				0.2	1.0		0.09
37.	Fresh Bottle gourd				0.2	1.0		0.31
38.	Dried Bottle gourd				0.2	1.0		0.27
39.	Fresh Curry				0.2	1.0		0.24

	leaves							
40.	Dried Curry leaves				0.2	1.0		0.13
41.	Fresh green amaranthus				0.2	1.0		0.32
42.	Dried Green amaranthus				0.2	1.0		0.30
43.	Fresh Red amaranthus				0.2	1.0		0.34
44.	Dried Red amaranthus				0.2	1.0		0.28
45.	Fresh Bitter gourd				0.2	1.0		0.32
46.	Dried Bitter gourd				0.2	1.0		0.23
47.	FreshBrinjal				0.2	1.0		0.40
48.	DriedBrinjal				0.2	1.0		0.30
49.	Fresh Carrot				0.2	1.0		0.35
50.	Dried Carrot				0.2	1.0		0.16
51.	Fresh Onion				0.2	1.0		0.55
52.	Dried Onion				0.2	1.0		0.52
53.	Fresh Broccoli				0.2	1.0		0.23
54.	Dried Broccoli				0.2	1.0		0.16
55.	Fresh Ivy gourd				0.2	1.0		0.55
56.	Dried Ivy gourd				0.2	1.0		0.52
57.	Fresh Mushroom				0.2	1.0		0.56
58.	Dried Mushroom				0.2	1.0		0.37
59.	Fresh Elephant foot yam				0.2	1.0		0.38
60.	Dried Elephant foot yam				0.2	1.0		0.32
61.	FreshGreen beans				0.2	1.0		0.46
62.	DriedGreen beans				0.2	1.0		0.42
63.	FreshMint				0.2	1.0		0.54
64.	DriedMint				0.2	1.0		0.52
65.	Fresh Lemon tea leaves				0.2	1.0		0.11
66.	Dried Lemon tea leaves				0.2	1.0		0.10

67.	Fresh marigold				0.2	1.0		0.25
68.	Dried marigold				0.2	1.0		0.18

Table 14: Observation for determination of carbohydrates.

Concentration of carbohydrates (µg /ml) by graph

Sample	Concentration of glucose (µg/ml)
1) Fresh chikoo	162
2) Dried chikoo	84
3) Fresh Mango	239
4) Dried mango	213
5) Fresh Guava	213
6) Dried Guava	210
7) Fresh pineapple	213
8) Dried pineapple	175
9) Fresh Tomato	130
10) Dried Tomato	50
11) Fresh Pumpkin	170
12) Dried Pumpkin	165
13) Fresh Lemon	125
14) Dried Lemon	80
15) Fresh Gooseberry	126
16) Dried Gooseberry	120
17) Fresh Fenugreek	125
18) Dried Fenugreek	110
19) Fresh Green chilli	240
20) Dried Green chilli	205
21) Fresh Ginger	132
22) Dried Ginger	130
23) Fresh Beetroot	229
24) Dried Beetroot	220
25) Fresh Sweet potato	220
26) Dried Sweet potato	125
27) Fresh Spinach	70
28) Dried Spinach	35
29) Fresh Coriander	140
30) Dried Coriander	40
31) Fresh Bottle Gourd	132
32) Dried Bottle Gourd	130
33) Fresh Curry leaves	120
34) Dried Curry leaves	75
35) Fresh Green amaranthus	160
36) Dried Green amaranthus	133
37) Fresh Red amaranthus	160
38) Dried Red amaranthus	130

39) Fresh Bitter gourd	160
40) Dried Bitter gourd	124
41) Fresh Brinjal	200
42) Dried Brinjal	132
43) Fresh Carrot	167
44) Dried Carrot	80
45) Fresh Onion	239
46) Dried Onion	110
47) Fresh Broccoli	112
48) Dried Broccoli	80
49) Fresh Ivy gourd	239
50) Dried Ivy gourd	230
51) Fresh Mushroom	240
52) Dried Mushroom	167
53) Fresh Elephant foot yam	170
54) Dried Elephant Foot yam	160
55) Fresh Green beans	210
56) Dried Green beans	200
57) Fresh Mint	220
58) Dried Mint	210
59) Fresh Lemon tea leaves	42
60) Dried Lemon tea leaves	39
61) Fresh Marigold	125
62) Dried Marigold	85

Table 15: Concentration of carbohydrates (µg /ml) by graph.

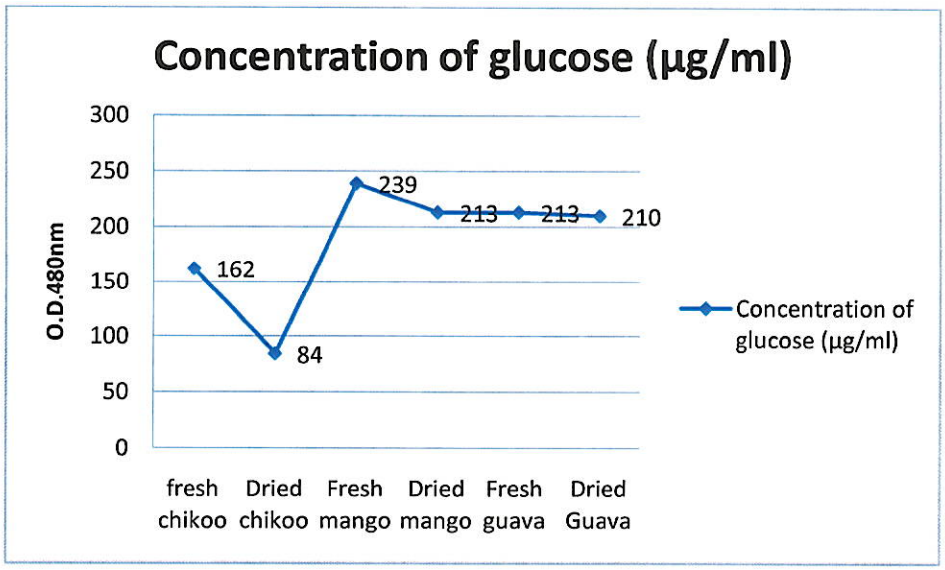

Figure 25: Graphical representation of concentration of glucose

8.4 Observations for antioxidant activity:

Conc.of standard (mg/ml)	Volume of standard (ml)	Volume of methanol (ml)	Total volume (ml)	Amount of DPPH (ml)	Incubate in dark condition for 30min	O.D. at 520 nm
Blank	-	5.0	5.0	3.0		0.00
0.2	1.0	4.0	5.0	3.0		0.20
0.4	2.0	3.0	5.0	3.0		0.22
0.6	3.0	2.0	5.0	3.0		0.25
0.8	4.0	1.0	5.0	3.0		0.27
1.0	5.0	-	5.0	3.0		0.28
Control	-	-	-	3.0		0.58
Fresh Chikoo			1.0	3.0		0.01
Dried Chikoo			1.0	3.0		0.10
Fresh Raw mango			1.0	3.0		0.01
Dried Raw mango			1.0	3.0		0.02
Fresh Guava			1.0	3.0		0.01
Dried Guava			1.0	3.0		0.03
Fresh Pineapple			1.0	3.0		0.09
Dried Pineapple			1.0	3.0		0.22
Fresh Tomato			1.0	3.0		0.01
Dried Tomato			1.0	3.0		0.02
Fresh Pumpkin			1.0	3.0		0.04
Dried Pumpkin			1.0	3.0		0.11

Fresh Lemon			1.0	3.0		0.03
Dried Lemon			1.0	3.0		0.22
Fresh Gooseberry			1.0	3.0		0.10
Dried Gooseberry			1.0	3.0		0.16
Fresh Fenugreek			1.0	3.0		0.12
Dried Fenugreek			1.0	3.0		0.08
Fresh Green Chilli			1.0	3.0		0.01
Dried Green Chilli			1.0	3.0		0.11
Fresh Ginger			1.0	3.0		0.07
Dried Ginger			1.0	3.0		0.08
Fresh Beetroot			1.0	3.0		0.05
Dried Beetroot			1.0	3.0		0.22
Fresh Sweet potato			1.0	3.0		0.11
Dried Sweet potato			1.0	3.0		0.08
Fresh Spinach			1.0	3.0		0.16
Dried Spinach			1.0	3.0		0.18
Fresh Coriander			1.0	3.0		0.07
Dried Coriander			1.0	3.0		0.10
Fresh Bottle gourd			1.0	3.0		0.05
Dried Bottle gourd			1.0	3.0		0.24

Fresh Curry leaves			1.0	3.0		0.11
Dried Curry leaves			1.0	3.0		0.09
Fresh Green amaranthus			1.0	3.0		0.12
Dried Green amaranthus			1.0	3.0		0.15
Fresh Red amaranthus			1.0	3.0		0.06
Dried Red amaranthus			1.0	3.0		0.10
Fresh Bitter gourd			1.0	3.0		0.10
Dried Bitter gourd			1.0	3.0		0.14
Fresh Brinjal			1.0	3.0		0.06
Dried Brinjal			1.0	3.0		0.08
Fresh Carrot			1.0	3.0		0.06
Dried Carrot			1.0	3.0		0.07
Fresh Onion			1.0	3.0		0.08
Dried Onion			1.0	3.0		0.18
Fresh Broccoli			1.0	3.0		0.09
Dried Broccoli			1.0	3.0		0.26
Fresh Ivy gourd			1.0	3.0		0.01
Dried Ivy gourd			1.0	3.0		0.09
Fresh Mushroom			1.0	3.0		0.09
Dried Mushroom			1.0	3.0		0.10

Fresh Elephant foot yam			1.0	3.0		0.12
Dried Elephant foot yam			1.0	3.0		0.18
Fresh Green beans			1.0	3.0		0.16
Dried Green beans			1.0	3.0		0.18
Fresh Mint			1.0	3.0		0.03
Dried Mint			1.0	3.0		0.02
Fresh Lemon tea leaves			1.0	3.0		0.12
Dried Lemon tea leaves			1.0	3.0		0.06
Fresh Marigold			1.0	3.0		0.07
Dried Marigold			1.0	3.0		0.10

Table 16: Observation for determination of antioxidant activity.

Calculation:

Formula : % Scavenging activity= $(Abs_{control} - Abs_{sample}) / Abs_{control} \times 100$

Sample	% Scavenging activity
1) Fresh Chikoo	98.27%
2) Dried Chikoo	40.75%
3) Fresh Raw mango	98.27%
4) Dried Raw mango	54.55%
5) Fresh Guava	98.27%
6) Dried Guava	52.81%
7) Fresh Pineapple	42.48%
8) Dried Pineapple	20.08%
9) Fresh Tomato	98.27%
10) Dried Tomato	54.55%
11) Fresh Pumpkin	93.10%
12) Dried Pumpkin	81.03%
13) Fresh Lemon	94.82%
14) Dried Lemon	20.06%
15) Fresh Gooseberry	40.75%

16) Dried Gooseberry	30.41%
17) Fresh Fenugreek	79.31%
18) Dried Fenugreek	40.75%
19) Fresh Green chilli	98.27%
20) Dried Green Chilli	81.03%
21) Fresh Ginger	45.93%
22) Dried Ginger	44.20%
23) Fresh Beetroot	49.37%
24) Dried Beetroot	20.06%
25) Fresh Sweet potato	81.03%
26) Dried Sweet potato	44.20%
27) Fresh Spinach	30.41%
28) Dried Spinach	26.96%
29) Fresh Coriander	45.93%
30) Dried Coriander	40.75%
31) Fresh Bottle gourd	49.37%
32) Dried Bottle gourd	16.62%
33) Fresh Curry leaves	81.03%
34) Dried Curry leaves	42.48%
35) Fresh Green amaranthus	79.31%
36) Dried Green amaranthus	32.13%
37) Fresh Red amaranthus	47.65%
38) Dried Red amaranthus	40.75%
39) Fresh Bitter gourd	40.75%
40) Dried Bitter gourd	33.86%
41) Fresh Brinjal	47.65%
42) Dried Brinjal	44.20%
43) Fresh Carrot	47.65%
44) Dried Carrot	45.93%
45) Fresh Onion	44.20%
46) Dried Onion	26.96%
47) Fresh Broccoli	42.48%
48) Dried Broccoli	13.17%
49) Fresh Ivy gourd	98.27%
50) Dried Ivy gourd	42.48%
51) Fresh Mushroom	42.48%
52) Dried Mushroom	40.75%
53) Fresh Elephant foot yam	79.31%
54) Dried Elephant foot yam	26.96%
55) Fresh Green beans	30.41%
56) Dried Green beans	26.96%
57) Fresh Mint	94.82%
58) Dried Mint	54.55%
59) Fresh Lemon tea leaves	79.31%
60) Dried Lemon tea leaves	47.65%

61) Fresh Marigold	45.93%
62) Dried Marigold	40.75%

Table 17:Calculation of percentage scavenging activity.

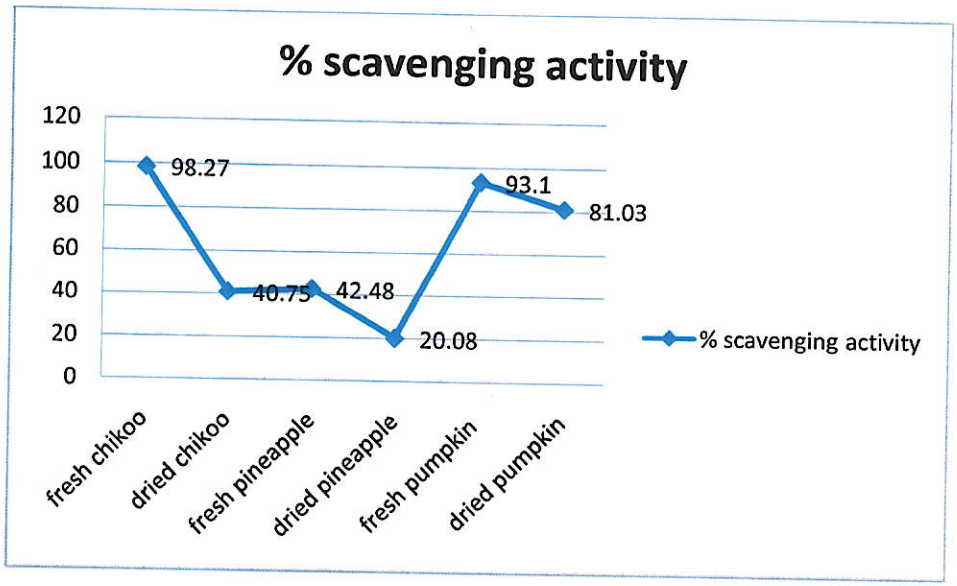

Figure 26: Graphical representation of % scavenging activity of some fruits.

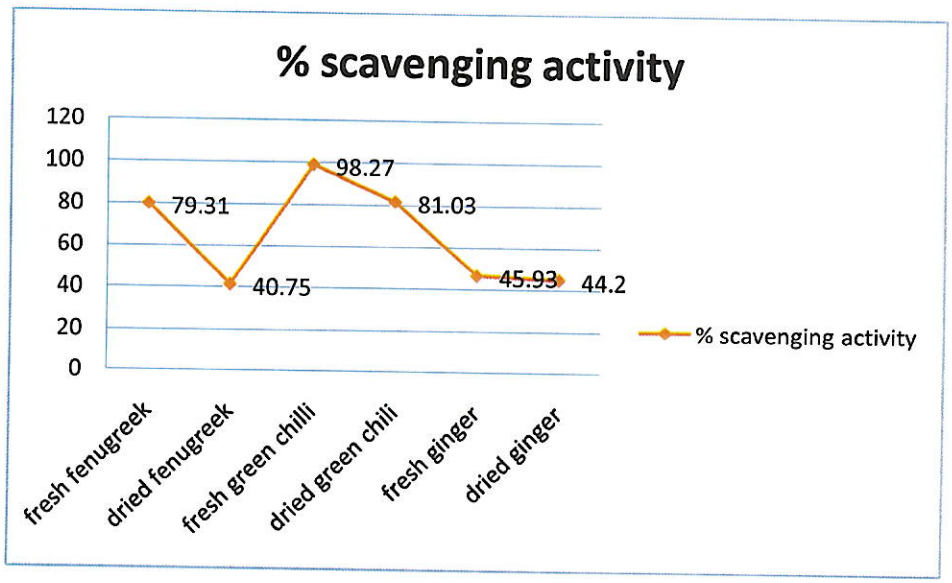

Figure 27: Graphical representation of % scavenging activity of some vegetables

8.5 Observation for determination of vitamin C by DCPIP:

Figure 28: Standard ascorbic acid

Titration Readings for Standard ascorbic acid:

1) Solution in conical flask- 5ml of std.ascorbic acid + 10 ml of 4%oxalic acid
2)bBurette solution : 2,6-dichlorophenolindophenol (DCPIP)

	I	II	III	C.B.R
Initial	0.00 ml	0.00 ml	0.00 ml	C.B.R.= 7.5 ml
Final	7.5 ml	7.3 ml	7.5 ml	
Difference	7.5 ml	7.3 ml	7.5 ml	

Table 18: Observation for standard ascorbic acid for determination of Vit C.

Titration reading for samples :

1) Solution in conical flask: 5ml of sample + 10ml of 4% oxalic acid.
2) Burette solution : 2,6-dichlorophenolindophenol (DCPIP)

Sample	Burette reading (C.B.R.) (ml)
1) Fresh Chikoo	6.0
2) Dried Chikoo	1.2
3) Fresh Raw mango	7.0
4) Dried Raw mango	1.0
5) Fresh Guava	2.6
6) Dried Guava	1.0
7) Fresh Pineapple	1.8
8) Dried Pineapple	0.6
9) Fresh Tomato	2.3
10) Dried Tomato	1.2

11) Fresh Pumpkin	2.5
12) Dried Pumpkin	1.9
13) Fresh Lemon	3.1
14) Dried Lemon	0.5
15) Fresh Gooseberry	3.4
16) Dried Gooseberry	2.6
17) Fresh Fenugreek	3.8
18) Dried Fenugreek	2.0
19) Fresh Green chilli	1.5
20) Dried Green Chilli	0.8
21) Fresh Ginger	1.6
22) Dried Ginger	0.5
23) Fresh Beetroot	3.8
24) Dried Beetroot	2.2
25) Fresh Sweet potato	2.6
26) Dried Sweet potato	1.0
27) Fresh Spinach	2.0
28) Dried Spinach	1.8
29) Fresh Coriander	0.8
30) Dried Coriander	0.4
31) Fresh Bottle gourd	2.6
32) Dried Bottle gourd	1.0
33) Fresh Curry leaves	0.9
34) Dried Curry leaves	0.8
35) Fresh Green amaranthus	3.1
36) Dried Green amaranthus	1.8
37) Fresh Red amaranthus	2.9
38) Dried Red amaranthus	0.9
39) Fresh Bitter gourd	3.6
40) Dried Bitter gourd	1.2
41) Fresh Brinjal	2.2
42) Dried Brinjal	1.0
43) Fresh Carrot	5.4
44) Dried Carrot	2.6
45) Fresh Onion	0.8
46) Dried Onion	0.2
47) Fresh Broccoli	3.9
48) Dried Broccoli	2.0
49) Fresh Ivy gourd	2.8
50) Dried Ivy gourd	1.0
51) Fresh Mushroom	1.0
52) Dried Mushroom	0.8
53) Fresh Elephant foot yam	1.8
54) Dried Elephant foot yam	0.6
55) Fresh Green beans	1.5

56) Dried Green beans	0.4
57) Fresh Mint	0.7
58) Dried Mint	0.6
59) Fresh Lemon tea leaves	3.6
60) Dried Lemon tea leaves	1.8
61) Fresh Marigold	1.2
62) Dried Marigold	0.5

Table 19: Observation for determination of vitamin C. for fruits, vegetables and flower.

Calculation:

$$\text{Amount of ascorbic acid content (gm\%)} = \frac{500 \times V_2 \times 25 \times 100}{V_1 \times 5 \times 5}$$

Where ,

1) 500= µgof standard ascorbic acid taken for titration,
2) V_1= volume of dye consumed by std. ascorbic acid,
3) V_2= volume of dye consumed by 5ml of test sample,
4) 25 corresponds to total volume of extract,
5) 100 = Ascorbic acid content/100g of the sample,
6) 5= weight of sample taken for extraction,
7) 5 = volume of test sample taken for titration.

Sample	Amount of ascorbic acid content (gm%)
1) Fresh Chikoo	40000
2) Dried Chikoo	8000
3) Fresh Mango	4666
4) Dried Mango	666
5) Fresh Guava	1733
6) Dried Guava	666
7) Fresh Pineapple	12000
8) Dried Pineapple	4000
9) Fresh Tomato	15,33.3
10) Dried Tomato	8000

11) Fresh Pumpkin	16666
12) Dried Pumpkin	1266
13) Fresh Lemon	2066.6
14) Dried Lemon	333.3
15) Fresh Gooseberry	2266.6
16) Dried Gooseberry	17333
17) Fresh Fenugreek	2533
18) Dried Fenugreek	13333
19) Fresh Green chilli	10000
20) Dried Green chilli	5333
21) Fresh Ginger	10,66.6
22) Dried Ginger	3,33.3
23) Fresh Beetroot	25,33.3
24) Dried Beetroot	1733.3
25) Fresh Sweet potato	4666.6
26) Dried Sweet potato	666
27) Fresh Spinach	13333.3
28) Dried Spinach	12000
29) Fresh Coriander	5333.3
30) Dried Coriander	2666.3
31) Fresh Bottle Gourd	1733.3
32) Dried Bottle Gourd	666.6
33) Fresh Curry leaves	6000
34) Dried Curry leaves	5333.3
35) Fresh Green amaranthus	20,66.6
36) Dried Green amaranthus	1200
37) Fresh Red amaranthus	19,333.3
38) Dried Red amaranthus	6000
39) Fresh Bitter gourd	24000
40) Dried Bitter gourd	8000
41) Fresh Brinjal	1466.6
42) Dried Brinjal	666.6
43) Fresh Carrot	36,000
44) Dried Carrot	1733.3
45) Fresh Onion	5333.3
46) Dried Onion	1333.3
47) Fresh Broccoli	26,000
48) Dried Broccoli	1333.3
49) Fresh Ivy gourd	1866.6
50) Dried Ivy gourd	666.6
51) Fresh Mushroom	666.6
52) Dried Mushroom	5,33.3
53) Fresh Elephant foot yam	12000
54) Dried Elephant Foot yam	4000
55) Fresh Green beans	10,000

56) Dried Green beans	2666.6
57) Fresh Mint	4666.6
58) Dried Mint	4000
59) Fresh Lemon tea leaves	24,000
60) Dried Lemon tea leaves	12,000
61) Fresh Marigold	8000
62) Dried Marigold	333.3

Table 20: Calculation for amount of ascorbic acid (gm%)

9. RESULTS & DISCUSSION:

Identification & estimation of nutrition content of different fruits and vegetable sample was carried out qualitatively, colorimetric&titrimetric analysis (nutrients like, protein, calcium, vitamins and carbohydrates were estimated)

Results obtained from the studies revealed that the dried fruits and vegetable sample contain less amount of nutrition content or absence of certain nutrient content. The nutrient content varied from different sample to sample both fresh as well as dried.

For protein content :

The protein in different fruits, vegetables as well as flower was estimated qualitatively in alkaline condition. There was difference in protein content of fruits and vegetables in fresh and dried samples. Most of the fresh sample showed presence of protein by giving bluish violet colour on addition of copper sulphate solution. Few dried samples showed presence of protein while others showed absence of protein by forming orange or green colour on addition alkaline copper sulphate solution. Fresh samples like Guava, Pumpkin, Gooseberry, Fenugreek, Ginger, Coriander, Curry leaves, Green & Red amaranthus, Brinjal, showed absence of protein. Dried samples likeChikoo, Mango, Guava, Pineapple, Pumpkin, Gooseberry, Fenugreek, Green chilli, Ginger, Beetroot, Spinach, Coriander, Bottle gourd , Curry leaves,Green & Red amaranthus, Brinjal, Onion, Ivy gourd, Mushroom, Green beans, Mint, Lemon tea leaves showed absence of protein.

For calcium content:

The calcium content in different fruits , vegetables as well as flower was estimated quantitatively by precipitation reaction. There was difference in calcium content of fruits and vegetables in fresh and dried samples. Most of the fresh samples showed presence of calcium by giving white precipitate. Few dried samples showed presence of calcium. Fresh samples that showed absence of calcium were Fenugreek, Beetroot, Spinach,Red amaranthus ,Brinjal, Mint ,Marigold. Dried samples that showed absence of calcium were Tomato, Lemon, Fenugreek, Beetroot, Spinach, Coriander, Red amaranthus,Brinjal, Broccoli, Mint, Lemon tea and Marigold.

For carbohydrate content:

The carbohydrate (total sugars) in different fruits and vegetable sample was determined colorimetric analysis by phenol-sulfuric acid method. The concentration of sugar (µg/ml) was estimated graphically. The concentration of sugar (µg/ml) in fresh fruit samples was found to be approximately ranging from 125-239µg/ml and in dried fruit samples it was found to be 84-213µg/ml. The highest concentration of sugar was found in fresh and dried mango sample 239 µg/ml& 213 µg/ml respectively. The concentration of sugar (µg/ml) in fresh vegetable samples was found to be approximately ranging from 70-240 µg/ml and in dried vegetable samples it was found to be approximately 35-210 µg/ml. The highest concentration of sugar was found in fresh green chilli and mushroom 240 µg/ml and in dried mint 210 µg/ml. It was found that the fresh sample contain more concentration of sugar than the dried samples.

For antioxidant activity:

The antioxidant activity of fresh and dried fruits and vegetable samples was determined by DPPH method. The percentage scavenging activity of all the samples was calculated. The highest scavenging activity was found to be 98.27% in fresh samples of chikoo,mango, guava, tomato and in green chilli. The highest scavenging activity was found in dried sample of mango which was 54.55% which is less as compared to fresh samples. The antioxidant activity in fresh samples ranged from approximately 22% - 99% while in dried samples it ranged from 20%-55% . It showed the fresh samples of both fruits and vgetables have more scavenging or antioxidant activity as compared to dried samples.

Md.Jannatul Feradus, Zannatul Ferdous, Rubyat Jahan Sara, Md. Omar Faruque (2020) they revealed that fresh fruits and vegetables contain proficiency to trap free radicals i.e. good antioxidant activity while their studies revealed that guava can scavenge more free radicals compared to other fruits and vegetable sample.

For Vitamin C:

The amount of Vitamin C or ascorbic acid was determined by using DCPIP method. The amount of ascorbic acid was calculated in gram percent (gm %). The amount of ascorbic acid in fresh fruits was estimated approximately from 4000-40,000 gm%. The more ascorbic acid content was found in fresh pineapple sample and least in fresh gooseberry. The amount of ascorbic acid content in dried fruits was estimated approximately from 3000-20,000gm%. The more ascorbic acid content was found in dried guava and least in dried lemon. The amount of ascorbic acid in fresh vegetables was estimated approximately from 5000-28,000 gm%. The more ascorbic acid content was found in fresh carrot and least in fresh coriander. The amount of ascorbic acid content in dried vegetables was estimated approximately from 2,000-19,000 gm%. The more ascorbic acid content was found in dried green chilli and least in dried coriander. The ascorbic acid content in marigold was found to 8000gm% in fresh sample while 333.3gm% in dried sample. The fresh samplescontain more Vitamin C as compared to dried sample.

Deekshika B. , Praveena Lakshmi B, Hemant Singuluri and M.K. Sukumaran (2015)revealed the fruits and vegetables are good source of vitamin C and citrus fruits are alternative source of vitamin C if there is scarcity of other fruits and vegetables

10.CONCLUSIONS:

Finally, it is concluded that aim of this project work is to determine and compare the nutrient content of different fruits and vegetables (fresh and dried) forming purpose with respect to health benefits of an individual. Different fresh fruits and vegetables were collected from market of Palghar and Boisar. The fresh samples were dried using solar drier AKRUTI center, BARC, Boisar. The results revealed that the fresh fruit and vegetable samples contain more nutrients as compared to dried samples. The results also revealed that some fresh sample showed absence of proteins or calcium which might be due to the fruits and vegetables may be grown with the help of chemical fertilizers which reduced the nutritional value of those fruits and vegetables which will directly affect the health of individual consuming it.

Also the study reported that the dried fruits and vegetables containing lesser nutrients can affect health of an individual if consumed frequently. Some samples also showed similarity with respect to their nutrient content. Based on the study it can be concluded that fresh fruits and vegetable sample ate more suitable for consumption while the consumption of dried or fruits and vegetables should be avoided.

Therefore, this study helped to determine which food should be included in diet and what should be avoided in order to lead a healthy life.

11.FUTURE PROSPECTUS:

In India, people are suffering from various diseases, disorders, or ill-condition due to improper diet. The number people affected due to diseases is increasing day by day this is mainly due lifestyle and consumption of junk food. It can be prevented by inculcating change in lifestyle and diet habits i.e. by including a healthy and balanced diet. This healthy and balanced diet includes intake of fresh fruits and vegetables that are available in market and depending upon the need and age of individual. Frequent consumption of preserved or dried foods should be avoided.

The fruits and vegetables that are rich in calcium can be included in the the diet of an individual having calcium deficiency or osteoporosis. The fruits and vegetables rich in protein can be included in diet of old people in order to overcome weakness arising due to age. Carbohydrates should be included in diet as it provides with glucose which supports body for its functions and metabolism. The fruits and vegetables with potential source of antioxidants should be included in diet in order to reduce the rate of free radicals induced diseases and ensure a healthy life.

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ISOLATION, CHARACTERIZATION AND OPTIMISATION OF CELLULOSE DEGRADING BACTERIA

A PROJECT SUBMITTED TO THE

University of Mumbai

TOWARDS PARTIAL FULFILMENT OF
THE DEGREE OF
MASTERS OF SCIENCE IN BIOTECHNOLOGY

UNDER THE GUIDANCE OF
PROF. ISHWARI N. MEHTA

SUBMITTED BY
MS. KAJOL ANIL KHILARE

DEPARTMENT OF BIOTECHNOLOGY SONOPANT DANDEKAR
SHIKSHAN MANDALI COLLEGE, PALGHAR, 401404.
M.Sc. BIOTECHNOLOGY (2022-23)

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(NAAC Reaccredated 'B' Grade)

Ref. No. :

Date : 10/07/2023

CERTIFICATE

The work described in this project entitled "Isolation, Characterization and optimization of Cellulose Degrading bacteria."

has been carried out independently by Kajol Anil Khilare Exam Seat no. 4540327 with the help of my support and encouragement. I certify that this is a bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

10/07/23

Signature of Guide

Signature of H.O.D

Co-ordinator
Department of Biotechnology
Sonopant Dandekar College, Palghar

Signature of Principal

13/07/23 13/7/2023

Signature of Examiner

DECLARATION

I, **KAJOL ANIL KHILARE,** Student of M.Sc. Part 2 Biotechnology hereby declare that the project entitled **"ISOLATION, CHARACTERIZATION AND OPTIMISATION OF CELLULOSE DEGRADING BACTERIA"** Submitted by me for the academic year 2022 -2023, is based on the actual work carried out by me under the guidance of Prof. Ishwari N. Mehta, I further state that this work is original and no part has been presented for any degree, diploma or similar title of any University.

Date: 10/07/2023
Place: Palghar

Certified by,

10/07/23

Signature of the Guide
Prof. Ishwari N. Mehta
Assistant professor
Department of Biotechnology

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Cellulases in brewing industry :

Enzymes are an essential ingredient for the brewing sector, particularly during the malting and fermentation phases. β-Glucans present a significant challenge in brewing as they can reduce product yield, increase wort and beer viscosity, and cause beer haze. Cellulases are used to facilitate wort drain, improve beer filterability, reduce solution viscosity and prevent gel formation in order to increase malt extraction. The processes mainly involve the utilization of endoglucanases and β-glucosidases.

In winemaking, cellulases are utilized alongside other enzymes like hemicellulose and pectinase during maceration, a vital step in the process. The macerating process aims to obtain color, taste and phenols from grape skins that are predominantly composed of pectocellulose. However, the utilization of cellulases and hemicellulases in later phases has resulted in increased process performance compared with traditional use of pectinases. Cellulases provide a fine and stable wine which is easily filtered, thus reducing the duration needed for completion of the maceration process.

Cellulases in biofuel production:

Depletion of fossil fuels and the increasing demand of alternate sources for renewable energy have developed a huge interest in cellulase production. Cellulases have potential application in biofuels production. Bioconversion of lignocellulosic substrate using cellulases and other enzymes are the thirst area for the commercialization of biofuels. The cellulase preparation in biomass conversion processes is based on a number of its properties such as stability, product inhibition, synergism, and composition of lignocellulosic biomass etc. Cellulase enzymes can be used to convert the cellulose portion of nonfood biomass, such as agricultural waste and energy crops, into fermentable sugars for subsequent conversion to renewable fuels and chemicals **[15].**

RATIONALE

Cellulase plays a key role in the breakdown of cellulose, the most abundant organic compound in the world. Cellulose degrading bacteria are potential sources of this enzyme and can be utilized in various industrial processes, such as biofuel production, paper and textile industries, waste management, and bioremediation.

In order to exploit their potential for commercial use, it is important to isolate and optimize cellulose degrading bacteria. Currently, cellulases are applied in the food industry, animal feeding stuffs, pharmaceutical formulations, textile and garment products, detergent production, paper and pulp industries as well as agriculture and research purposes. The advent of molecular biotechnology has opened the way for the development of powerful expression systems for enzymes, and their engineering for improved performance as well as the optimization of each particular condition will be essential for reducing production costs.

This study aims to screen and isolate cellulose degrading bacteria from a soil sample. And its characterization by studying its morphological and biochemical characteristics. The maximum cellulose degrading bacteria is determined by estimating the amount of reducing sugars released by the DNSA method and optimizing its parameters, such as pH and temperature.

REVIEW OF LITERATURE

In the study carried out by Nandimath et.al (2016), 40 different bacterial isolates were isolated and screened from soil and were identified as *Pseudomonas sp.* and *Bacillus sp.* The maximum cellulase production occurred at 30^0 C at pH 5 by *Pseudomonas sp.*

Sethi et.al (2013), isolated 4 different bacterial strains from soil and identified them as Pseudomonas *fluorescens, Bacillus subtilis, E. coli,* and *Serratia marcescens* . The maximum cellulase production occurred at 40^0C at pH 10 with glucose as a carbon source and ammonium sulfate as a nitrogen source by *Pseudomonas fluorescens.*

In the work conducted by Islam and Roy (2018), 3 different bacterial strains were isolated and screened from sugar industry waste and were identified as *Paenibacillus sp., Bacillus sp.* and *Aeromonas sp.* The maximum cellulase production occurred at 40^0C at pH 7.0 in a medium containing 1% CMC by *Paenibacillus sp.*

Two different bacterial strains, *Bacillus subtilis CY5* and *Bacillus circulans TP3*, were found in the gastrointestinal tracts of *Mozambique tilapia, Oreochromis mossambicus*, and common carp, *Cyprinus carpio L.*, respectively, in the research done by Ray et al. in 2007. For both strains, the highest cellulase production occurred at a temperature 40^0C and at a pH range of 7.0 to 7.5.

Five different bacterial strains were isolated and screened from soil in Brazzaville by Lingouangou et.al (2022) and were identified as *Pantoea dispersa, Pseudomonas aeruginosa , Pseudomonas monteilii, Bacillus subtilis* and *Lysinibacillus fusiformis*. The optimum cellulase production occurred at 40°C at pH 8.

In the previous study done by Shah et.al (2021), a cellulose degrading bacteria was isolated from nearby zones and industrial areas and was identified as *Bacillus pumilus* . The maximum cellulase activity occurred at 37°C and at pH 4.

AIMS AND OBJECTIVES

AIM: To isolate, characterize and optimize the cellulose degrading bacteria from the soil.

OBJECTIVES:

1. Screening and isolation of cellulose degrading bacteria from the soil.
2. Morphological and biochemical characterization of cellulose degrading bacteria.
3. Determination of cellulase activity by the DNSA method.
4. Process Optimization of cellulase producing bacteria.

MATERIALS AND METHODS

1. SCREENING AND ISOLATION OF CELLULOSE DEGRADING BACTERIA:

Soils samples were collected from the SDSM college area, Palghar, India . Cellulose is isolated by serially diluting the soil sample using sterile saline and inoculated on CarboxyMethylCellulose (CMC) agar by spread plate method. Plates were incubated at room temperature for 24-48 hrs. After incubation to observe the zone of hydrolysis, plates were flooded with 10 ml of 0.1 % Congo red solution for 15 mins and then washed using 1 M NaCl solution .

Requirements:

1	Sample	Soil sample	1 gm
2	Media used	Sterile CarboxyMethylCellulose (CMC) Agar	100 ml
3	Reagents used	1. 0.1 % Congo red solution	50 ml
		2. 1 M NaCl	50 ml
4	Glasswares used	1. Sterile test tubes	6
		2. Sterile 10 ml pipettes	3
		3. Sterile 1 ml pipettes	1
		4. Clean and dry beaker	1
		5. Sterile petri dishes	6
5	Instruments used	1. Weighing balance	
		2. Autoclave set at 121^0C for 15 mins	
		3. pH meter	
6	Miscellaneous	1. Test tube stand	

		2. Sterile saline	50 ml
		3. Tripod stand	
		4. Cotton	
		5. Disinfectant	

Table 1: Requirement table for screening and isolation of cellulose degrading bacteria.

2. MORPHOLOGICAL AND BIOCHEMICAL CHARACERISTICS:

Selected bacterial isolates are initially characterize by Morphological characteristics , Gram staining , Sugar Fermentation , Gelatin Hydrolysis, Starch Hydrolysis , Indole Test , Methyl Red Test , Voges Proskauer Test , , Citrate Utilization and Triple Sugar Iron Test.

Requirements:

1	Sample	18 -24 hrs old culture suspension of Cellulose degrading bacterial isolate.
2	Media	CMC agar
3	Glasswares	Sterile petriplates
4	Miscellaneous	1. Nichrome loop
		2. Cotton
		3. Disinfectant

Table 2: Requirement table for morphological characteristics

- **GRAM STAINING :**

Principle: In Gram staining , bacteria are first stained with the primary stain Crystal Violet and then treated with mordant. Some bacteria will retain their primary stain after this, while others decolorize with alcohol. The cell walls of gram-positive bacteria are composed of a thick layer of peptidoglycan, a complex of protein and sugar, and have low lipid content. When alcohol is applied to the cells, the thick peptidoglycan layer dehydrates and shrinks, which seals the pores in the cell wall and prevents the stain from leaving the cell. As a result, the Crystal Violet-Iodine complex remains bound to the thick layer of peptidoglycan of gram-positive bacteria, and they appear blue or purple in color.

In contrast, cell walls of gram negative bacteria are composed of a small layer of peptidoglycan and a thick outer layer of lipids. CVIodine also penetrates the cell walls of gram negative bacteria, but the decolorizer breaks down the lipids in the cell wall, causing the CVIodine complex to be washed away. As a result, gram-negative bacteria appear colorless after decolorization. When the cells are re-stained with safranin, they take up the stain and appear red in color.

Requirements :

1	Sample	18 - 24 hrs old culture suspension of isolated cellulase producing bacteria
2	Reagents	1. Crystal violet
		2. Gram's Iodine
		3. 75 % alcohol
		4. Safranin
3	Glasswares	1. Clean and dry grease free slides
		2. Dropper
4	Miscellaneous	1. Nichrome loop

		2. Oil
		3. Microscope
		4. Filter paper
		5. Distilled water

Table 3:Requirement table for gram staining .

Protocol :

Take a loopful of 18 -24 hrs old culture suspension of cellulose degrading bacteria and make a smear on clean and dry grease free slide.

⇩

Heat fix the smear.

⇩

Flood the slide with the crystal violet for 1 minute.

⇩

Wash the slide with distilled water.

⇩

Add gram's iodine and keep it for 1 minute.

⇩

Decolourize using 75% alcohol for 30 seconds.

⇩

Counter stain using safranin and keep it for 1 minute.

⇩

Wash the slide using distilled water.

⇩

Discard the excess stain and let the slide air dry completely.

⇩

Place a drop of the oil on the slide. Observe under oil immersion lens (100X) of microscope.

- **SUGAR FERMENTATION TEST :**

Principle: When microorganisms ferment carbohydrates, they produce acids or acids with gas. Bacterial fermentation may result in the formation of specific end products depending on the types of organisms and substrates used to produce them. Lactic acid, formic acid, acetic acid, butyric acid, butyl alcohol, acetone, ethyl alcohol, carbon dioxide, hydrogen can be included in these end products.

The pH of the test medium will be reduced as acid production occurs during fermentation. The use of a pH indicator such as andrade's indicator can be used to detect this change in pH. Other pH indicators like bromocresol purple (BCP), bromothymol blue (BTB), and Phenol red can be used.The pH indicator undergoes a color change when enough acid has been released. It is important to note that bacteria are also capable of using the peptone in the medium and producing alkaline byproducts, which does not result in color change.

Durham tubes are inserted upside down into the test tube to detect gas production during fermentation. When the test organisms produce gases, they displace media inside the Durham tube and this causes an air bubble to form.

The bacteria may be divided into three groups based on the known characteristics of these reactions: Fermenters with only acid production, fermenters with acid and gas production, and non fermenters.

Requirements :

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	1. Sterile 1% Glucose broth with inverted durham's tube and andrade's indicator
		2. Sterile 1% Maltose broth with inverted durham's tube and

		andrade's indicator
		3. Sterile 1% Mannitol broth with inverted durham's tube and andrade's indicator
		4. Sterile 1% CarboxyMethyl Cellulose broth with inverted durham's tube and andrade's indicator
		5. Sterile 1% Cellulose broth with inverted durham's tube and andrade's indicator
3	Glasswares	Sterile test tubes
4	Instrument	Incubator set at 37^0 C
5	Miscellaneous	1. Nichrome loop
		2. Disinfectant
		3. Cotton
		4. Test tube stand

Table 4: Requirement table for sugar fermentation

Protocol :

Inoculate all the sugar fermentation broth with a loopful of 18-24 hrs old culture suspension of bacterial isolate.

⇩

Incubate at 37^0C for 24 - 48 hrs.

⇩

Observe the media for acid and gas production.

- **GELATIN HYDROLYSIS :**

Principle: The gelatin hydrolysis test is a method used to detect the presence of gelatinases in certain bacteria. Gelatinases are enzymes produced by some bacteria that can hydrolyze or digest the gelatin.This process occurs in two steps. First ,the gelatinase breaks down the gelatin into polypeptides, and then the polypeptides are broken down into amino acids.

To perform this test, a nutrient gelatin medium is used, which is a mixture of peptone , beef extract, and gelatin. Gelatin serves as both a substrate for gelatinase activity and as a means of solidifying the medium. When a gelatinase-positive bacterium is stab-inoculated into the nutrient gelatin medium, the secreted gelatinases will break down the gelatin, resulting in liquefaction of the medium. The medium will remain liquid even after being placed in a refrigerator or ice bath as the gelatin is digested and can no longer solidify. On the other hand, if a gelatinase negative bacterium is inoculated into the medium, the gelatin will not be digested, and the medium will remain solid after cooling.

Requirements :

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Nutrient Gelatin Agar deep
3	Glasswares used	Sterile petri plates
4	Instrument	Incubator set at 37^0 C
5	Miscellaneous	1. Nichrome loop
		2. Disinfectant
		3. Cotton

Table 5: Requirement table for gelatin hydrolysis

Protocol :

Inoculate a loopful of 18-24 hrs old culture suspension of bacterial isolate in the sterile nutrient gelatin agar deep.

⇩

Incubate at 37^0 C for 24 - 48 hrs.

⇩

Gelatin usually liquefies at 28°C and above, so to confirm that liquefaction was due to gelatinase activity, the tubes are dipped in an ice bath for 15 to 30 minutes.

⇩

Afterward, tubes are tilted to observe if gelatin has been hydrolyzed.

⇩

Hydrolyzed gelatin results in a fluid medium even if it is exposed to a cooling temperature ice bath while the uninoculated control medium will remain solid.

- **STARCH HYDROLYSIS TEST :**

Principle: Bacteria have the capability to produce extracellular enzymes to facilitate chemical reactions outside of the cell. These enzymes are used to break down nutrient sources such as starch, which is too large to be absorbed through the cell membrane, into smaller molecules that can be transported into the cell through diffusion.

In order to test the hydrolysis of starch, the bacteria are grown on agar plates containing starch. In the medium, the bacteria capable of hydrolyzing starch will do so, especially in regions surrounding their growth. Since no colour change occurs in the medium when starch is hydrolyzed, iodine solution should be used as an indicator on a plate after incubation. When non hydrolysed starch reacts with iodine, a dark blue colour is observed when the hydrolyzed end products do not form this complex. As a result, there are clear zones around the colonies that hydrolyze starch, while the rest of the plate shows a dark blue color due to the formation of the iodine- starch complex.

Requirements:

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Starch Agar
3	Reagents	Gram's Iodine
4	Glasswares used	Sterile petri plates
5	Instrument	Incubator set at 37^0 C
6	Miscellaneous	1. Nichrome loop
		2. Disinfectant
		3. Cotton

Table 6: Requirement table for starch hydrolysis

Protocol:

Streak the loopful of 18-24 hrs old culture suspension of bacterial isolate on the Sterile Starch agar plate.

⇩

Incubate the plates at 37^0 C for 24 - 48 hrs.

⇩

After incubation, flood the plates with gram's iodine to observe the zone of hydrolysis.

• INDOLE TEST:

Principle: Tryptophan is an amino acid that can be subjected to deamination and hydrolysis by bacteria expressing the enzyme tryptophanase. Through an intermediate molecule called indole pyruvic acid, indole is produced via reductive deamination of tryptophan. The deamination reaction occurs which is catalyzed by tryptophanase enzyme where the removal of the amine (-NH2) group from the tryptophan molecule takes place leading to the formation of indole, pyruvic acid, ammonium (NH4+), and energy as the end products . To trigger this reaction, a coenzyme pyridoxal phosphate must be present

When Kovac's Reagent is added to indole, the resulting solution changes from yellow to cherry red. The Kovac reagent consists of hydrochloric acid, p- dimethylamino benzaldehyde and ethyl alcohol. Due to the insolubility of amyl alcohol in water, the red colour forms in an oily layer on the top of the broth.

Requirements:

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Tryptone Broth (5ml)
3	Reagents	Kovac's reagent
4	Glasswares	Sterile test tubes
5	Instrument	Incubator set at 37^0 C
6	Miscellaneous	1. Nichrome loop
		2. Dropper
		3. Disinfectant
		4. Cotton

Table 7: Requirement table for indole test

Protocol:

Inoculate loopful of 18-24 hrs old culture suspension of bacterial isolate in the sterile Tryptone Broth.

⇩

Keep one broth uninoculated as a control.

⇩

Incubate at 37^0C for 24 - 48 hrs.

⇩

After incubation, add 4 - 5 drops of Kovac's reagent in the tryptone broth and Observe the colour change .

- **METHYL RED (MR) TEST:**

Principle: A method to determine whether an organism is capable of producing and maintaining stable acid end products from glucose fermentation is the MR test, also known as the Methyl Red test. It is usually performed in parallel with the VP test in MRVP Broth, because they are physiologically related. All *Enterobacteriaceae* family members undergo glucose conversion by the Embden Meyerhof pathway, but bacteria also have a way of converting pyruvic acid through two distinct pathways. Organisms that metabolize pyruvic acid through the mixed acid pathway produce more acid end products, such as acetic acid, lactic acid, and formic acid, and maintain an acidic environment. In order to detect mixed acid fermentations that result in lowering the pH of the water, a methyl red test is used. After incubation, the MR indicator is added, which is red at pH 4.4 and yellow at pH 6.2. After the addition of the pH indicator, the broth medium will remain red if the organism produces a large amount of organic acids such as formic acid, acetic acid, lactic acid, and succinic acid from glucose fermentation. The initial fermentation products are broken down by MR negative organisms through decarboxylation to produce neutral acetyl methyl carbinol, acetoin, which results in reduced acidity in the medium and increases pH neutrality to 6.0 or higher. The broth medium turns yellow for organisms that do not produce acid end products, giving a negative test result.

Requirements:

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Glucose Phosphate Broth (5ml)
3	Reagents	Methyl Red
4	Glasswares	Sterile test tubes
5	Instrument	Incubator set at 37^0 C
6	Miscellaneous	1. Nichrome loop
		2. Dropper
		3. Disinfectant
		4. Cotton

Table 8: Requirement table for methyl red test

Protocol:

Inoculate loopful of 18-24 hrs old culture suspension of bacterial isolate in the sterile Glucose Phosphate Broth.

⇩

Keep one broth uninoculated as a control.

⇩

Incubate at 37^0 C for 24 - 48 hrs.

⇩

After incubation, add 4 - 5 drops of methyl red reagent in the glucose phosphate broth.

⇩

Observe the colour change.

• VOGES PROSKAUER (VP) TEST:

Principle: Acetylmethylcarbinol (acetoin) and butanediol are the neutral end products generated by organisms that uses the butylene glycol pathway. This pathway increases the pH towards neutrality (pH 6) and results in a higher final pH. Both butylene glycol and mixed acid pathways are known to be shown by most *Enterobacteriaceae,* although not both. The organisms producing acetylmethylcarbinol from glucose fermentation can be determined by Voges Proskauer test. In the presence of α-naphthol, strong alkali (40% KOH), and atmospheric oxygen, acetylmethylcarbinol is converted to diacetyl. First, the -naphthol is added since it enhances colour. Guanidine-containing substances found in the peptone in the broth combine with diacetyl to generate a pinkish-red polymer.

Requirements:

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Glucose Phosphate Broth (5ml)
3	Reagents	O'meara's reagent
4	Glasswares	Sterile test tubes
5	Instrument	Incubator set at 37^0 C
6	Miscellaneous	1. Nichrome loop
		2. Dropper
		3. Disinfectant
		4. Cotton

Table 9: Requirement table for VP test

Protocol: Inoculate loopful of 18-24 hrs old culture suspension of bacterial isolate in the sterile Glucose Phosphate Broth.

Keep one broth uninoculated as a control.

⇩

Incubate at 37^0C for 24 - 48 hrs.

⇩

After incubation, add 4 - 5 drops of O'meara's reagent in the glucose phosphate broth.

⇩

Observe the colour change.

- **CITRATE UTILIZATION TEST:**

Principle: Citrate agar is a medium used to determine if an organism can use citrate as a sole source of energy. Citrate serves as the single source of carbon in the medium while inorganic ammonium salts ($NH_4H_2PO_4$) serve as the sole source of nitrogen.

Bacteria that possess citrate-permease enzyme can grow on this medium by converting citrate to pyruvate which then enters into the metabolic cycle for the production of energy. The bacteria growth is indicative of the use of citrate as an intermediate metabolite in Krebs's cycle. The citrate metabolism by bacteria leads to a breakdown of ammonium salts into ammonia, which results in an increased alkaline content in the medium. The color of the bromothymol blue indicator changes from green to blue above pH 7.6, as a result of change in Ph. This color change is used as an indicator for the citrate utilization by the bacteria.

Requirements:

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Simmons Citrate slant (5ml)
3	Glasswares	Sterile test tubes
4	Instrument	Incubator set at 37^0 C

5	Miscellaneous	1. Nichrome loop
		2. Disinfectant
		3. Cotton

Table 10: Requirement table for citrate utilization test

Protocol:

Streak the loopful of 18-24 hrs old culture suspension of bacterial isolate on the sterile Simmons citrate slant.

⇩

Keep one slant uninoculated as a control.

⇩

Incubate at 37^0C for 24 - 48 hrs.

⇩

Observe the colour change of slant from green to blue.

- **TRIPLE SUGAR IRON TEST :**

Principle: In the presence of lactose (or sucrose) fermentation, a significant amount of acid is produced, resulting in a yellow color change of the phenol red indicator in both the butt and the slant of the medium. In the case of certain organisms, gases may be generated which could lead to bubbles or cracks in the medium.

If lactose is not fermented, but a small amount of glucose is fermented, the butt of the medium, which contains a higher concentration of glucose, will turn yellow due to the production of acid under oxygen-deficient conditions. The acid produced will be oxidized to carbon dioxide and water by the organism on the slant of the medium, where there is less glucose and the media is more aerated. As a result, the slant will be red indicating more alkaline and neutral pH.

Both the butt and the slant of the medium will remain red, if neither lactose/sucrose nor glucose is fermented. In addition, due to ammonia production from oxidative deamination of

ABSTRACT

Cellulose degrading bacteria was isolated from soil samples collected from the garden area of SDSM College, Palghar. The screening of the cellulose degraders was carried out by Spread plate technique on the CMC (Carboxymethyl Cellulose) medium. The bacteria isolated were found to be Gram positive cocci along with biochemical characterization. Process optimization of cellulose degrading bacteria was carried out by considering the parameters like temperature and pH. The maximum cellulase activity was observed at 25^0C at pH 8.0.

Keywords: Cellulose degrader, Carboxymethyl Cellulose, Optimization, pH , Temperature.

INTRODUCTION

Biomolecules derived from natural resources are playing a major role in manufacturing products needed for daily use **[3]**. Enzymes are one of those molecules that are produced by all living beings. These are normally referred to as biocatalysts. Recent trends of replacing hazardous chemicals in the industrial sector with a green chemistry approach have led to an ever-increasing demand for enzymes, such as their role in brewing, milk products, detergents, food and feed, pharmaceutical production or paper and pulp industry. In this context, to render the large-scale production of chemicals feasible,the availability of cost-effective substrates of the enzymes is very important. Cellulase is one of these enzymes which has the greatest widespread use.

Cellulose is the most abundant biomass on Earth **[12]**. It is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the biosphere. All cellulose is produced biosynthetically, mostly by algae and higher plants but also by certain bacteria, marine invertebrates, fungi, slime molds and amoebae **[17]**. Plants are the most abundant source of cellulose and are found as microfibrils ("2-20 nm" in diameter and "100-40,000 nm" in length) **[9]**. The microfibrils have a structural role in the cell wall, imparting strength and contributing to its size and shape. Plants synthesize about 4 x 10^9 tonnes of cellulose annually but this material does not accumulate because fungi and bacteria efficiently degrade all plant biomass to provide themselves with energy and carbon, ultimately recycling carbon dioxide into the ecosystem **[17].** It is also regarded as one of the most significant carbon sources on Earth, and both marine and terrestrial plants biosynthesize 0.85 x 10^{11} tonnes of it annually.

Cellulose is a homopolymer of d-glucose units linked by β-1,4 bonds , mainly degraded by cellulase enzymes which are commonly produced by bacteria and fungi. The cellulases can effectively hydrolyze cellulose into glucose units via the synergistic actions of the enzymes, known as endo-β-1,4 glucanase, cellobiohydrolase and β-D-glucosidase **[2].** The exoglucanase (CBH) acts on the ends of the cellulose chain and releases *β*-cellobiose as the end product; endoglucanase (EG) randomly attacks the internal *O*-glycosidic bonds, resulting in glucan chains of different lengths; and the *β*-glycosidases act specifically on the *β*-cellobiose

disaccharides and produce glucose. Although the mechanism of cellulose degradation by aerobic bacteria is similar to that of aerobic fungi, it is clear that anaerobic bacteria operate on a different system **[5]**. Only the synergy of the above three enzymes makes the complete cellulose hydrolysis to glucose or a thorough mineralization to H_2O and CO_2 possible **[1].**

Cellulase is synthesized by a number of microorganisms such as yeast, fungi and bacteria. It can be produced in large quantities by using fermentation technology. To overcome the increasing industrial demand for important cellulases, the global production has been improved by using fermentation technology and augmentation of downstream processing for the recovery of purified cellulases in large amounts . The industrial production of cellulases can be carried out in both solid state fermentation and submerged fermentation **[13]**.

The established approach for enhancing the enzyme productivity and cost reduction at industrial level involves the designing of experiments for the optimization of physical and factors. The production of cellulase including endoglucanase can be influenced by both physical parameters such as temperature, pH and moisture and chemical factors such as carbon, nitrogen and minerals. Among all chemical factors, carbon is the most important source that usually acts as a substrate and depends on the yield of enzymes. The substrate must be easily available and cost effective. The selection of appropriate and cheap carbon source, nutrient media, culture and optimization of conditions are required for the maximum production of Endoglucanase **[13]**.

Abundant availability of cellulose makes it an attractive raw material for producing many industrially important commodity products. Sadly, much of the cellulosic waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon. With the help of a cellulolytic system, cellulose can be converted to glucose which is a multi-utility product, in a much cheaper and biologically favorable process **[1]**.

Cellulose containing wastes may be agricultural, urban, or industrial in origin; sewage sludge might also be considered a source of cellulose since its cellulosic content provides the carbon needed for methane production in the anaerobic digestion of sludge. Agricultural wastes include crop residue, animal excreta and crop-processing wastes, slashing generated in logging, saw dust formed in timber production, and wood products in forestry originated activities. The

previous negative attitude in which wastes were viewed self-consciously as valueless and even offensive and for disposal only has been replaced in large part by a positive view in which wastes are recognized as raw materials of potential value **[12]**.

A large number of organisms utilize cellulose as their chief food source including fungi, bacteria, plants, and protists, as well as a wide range of invertebrate animals, such as insects, crustaceans, annelids, mollusks, and nematodes **[14].** A few microorganisms like *Xanthomonas, Actinomycetes, Clostridium species, Ruminococcus albus, Aspergillus species, Chaetomium species, Fusarium species, Methanobrevibacter ruminantium, Myrothecium species, Penicillium species, Butyrivibrio fibrisolvens, Bacteroides succinogenes, Acinetobacter species, Bacillus cereus, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeruginosa, Trichoderma species*, and so forth are accounted for in light of the capability of cellulase creation by degrading cellulose **[8].**

Microbial cellulases have shown their enormous potential in a wide range of industries over many decades. This class of enzymes is widely used in a variety of industry processes including biofuels production, food and wine biotechnology, paper manufacturing, biomass conversion, textile and clothing industries, applications for R&D as well as in medicine and agriculture.

Cellulases in Textile industry:

Cellulases are widely applied in numerous sectors because they are made from renewable resources and waste. They have a wide range of uses in textiles, detergents, and other biotechnology fields, with a recent concentration on the textile industry . Denim washing with cellulase is a common eco-friendly procedure for achieving a pleasing look and non-abrasiveness in cotton textures and denim. However, previous enzymatic denim washing methods used acid cellulase (*Trichoderma reesei*) and neutral cellulase (*Humicola isolens*), both of which had the problem of causing indigo color back staining on the cloth. Although it has been suggested that pH is the most important factor in preventing back stains, there have been no reports on the use of cellulase for denim washing under soluble circumstances. Under basic conditions, a soluble base stable endoglucanase from *alkalothermophilic* bacteria. *Thermomonospora* sp. (T-EG) was used for denim finishing . The textile industry meets one of humanity's most fundamental necessities while also contributing significantly to many

country's economic growth. The demand for textile materials is increasing as the population grows and per capita consumption of textiles rises **[4].**

Cellulases in food processing and animal feed industry :

Cellulases are widely used in food processing and animal feed production, mainly in combination with hemicellulases and pectinases. Their uses are varied; for example, they are used in production of fruit and vegetable juices, carotenoids and degradation of plant cell walls for wine and beer industries, among others. In the fruit juice production industry, cellulases produced by bacteria, such as *Bacillus* and *Paenibacillus*, besides other cellulases of fungal origin, are used as accessory enzymes for clarifying fruit juices. Moreover, cellulases in combination with pectinases degrade orange, sweet potato and carrot cell walls, so indirectly, cellulases are involved in the extraction of carotenoids, which are susceptible to be used in the food industry as coloring substances **[7].**

Cellulases are utilized in various ways, including improving the digestibility of cereal-based food and enhancing the nutritive value of forages in animal feed. In the context of monogastric animal feed, *Bacillus subtilis* cellulases can be employed to degrade soya grain hulls, thereby enriching their nutritional content. This application aims to provide higher-quality feed for monogastric animals.

Cellulases in paper and pulp industry:

In recent years, there has been a considerable increase in the use of Cellulases in the paper industry. Due to the fact that paper consists of natural polymers, namely cellulose, hemicelluloses, and lignin, microbial enzymes and organisms hold potential for their utilization in paper processing. The resulting pulps often have a high content of fines, mass, and stiffness in the mechanical pulping process, in which wood raw materials are refined and crushed. However, the application of cellulases in biomechanical pulping has led to significant energy savings (20–40%) during the refining process and improvements in the properties of hand sheets. In addition, combinations of cellulases (specifically endoglucanases I and II) and hemicellulases have been employed for the biomodification of fiber properties, aiming to enhance drainage and overcome challenges in paper processing before or after pulp beating. With a lower concentration of hydrolysis, the use of endoglucanases may reduce the thickness

of the pulp. They are also used in production of biodegradable cardboard, soft paper, such as towel and sanitary paper, as well as the removal of adhered paper.

Cellulases alone, or used in combination with xylanases, are beneficial for deinking of different types of paper wastes. Most applications proposed so far use cellulases and hemicellulases for the release of ink from the fiber surface by partial hydrolysis of carbohydrate molecules. It has been postulated that improvements in dewatering and deinking of various pulps result in the peeling of the individual fibrils and bundles, which have high affinity for the surrounding water and ink particles. Moreover, deinking using enzymes at acidic pH also prevents the alkaline yellowing, simplifies the deinking process, changes the ink particle size distribution, and reduces the environmental pollution. Although enzymatic deinking can lower the need for deinking chemicals and reduce the adverse environmental impacts of the paper industry, the excessive use of enzymes must be avoided, because significant hydrolysis of the fines could reduce the bondability of the fibers **[11].**

Cellulases in laundry and detergent industry:

Use of cellulases along with protease and lipase in the detergents is a more recent innovation in Laundry and detergent industry. Cellulase preparations capable of modifying cellulose fibrils can improve color brightness, feel, and dirt removal from the cotton blend garments. The industrial application of alkaline cellulases as a potential detergent additive is being actively pursued with a view to selectively contact the cellulose within the interior of fibers and remove soil in the interfibrillar spaces in the presence of the more conventional detergent ingredients. Nowadays, liquid laundry detergent containing anionic or nonionic surfactant, citric acid or a water soluble salt, protease, cellulose, and a mixture of propanediol and boric acid or its derivative has been used to improve the stability of cellulases. As most of the cellulose fibers in the modern textile industry enzymes are used increasingly in the finishing of fabrics and clothes are arranged as long, straight chains of some small fibers can protrude from the yarn or fabric. The cellulases are applied to remove these rough protuberances for a smoother, glossier, and brighter-colored fabric **[11].**

amino acids, the slant may show a deeper red purple color. The important thing to point out is that this change in colour may be influenced by the peptone which is the major component of TSI's agar medium.

The formation of black-colored ferrous sulfide can be observed in the presence of hydrogen sulfide (H_2S) production.

Requirements:

1	Sample	18-24 hrs old culture suspension of bacterial isolate
2	Media used	Sterile Triple sugar iron slant (5ml)
3	Glasswares	Sterile test tubes
4	Instrument	Incubator set at 37^0 C
5	Miscellaneous	1. Nichrome loop
		2. Disinfectant
		3. Cotton

Table 11: Requirement table for TSI test

Protocol:

Streak the loopful of 18-24 hrs old culture suspension of bacterial isolate on the sterile Triple sugar iron slant.

⇩

Keep one slant uninoculated as a control.

⇩

Incubate at 37^0C for 24 - 48 hrs.

⇩

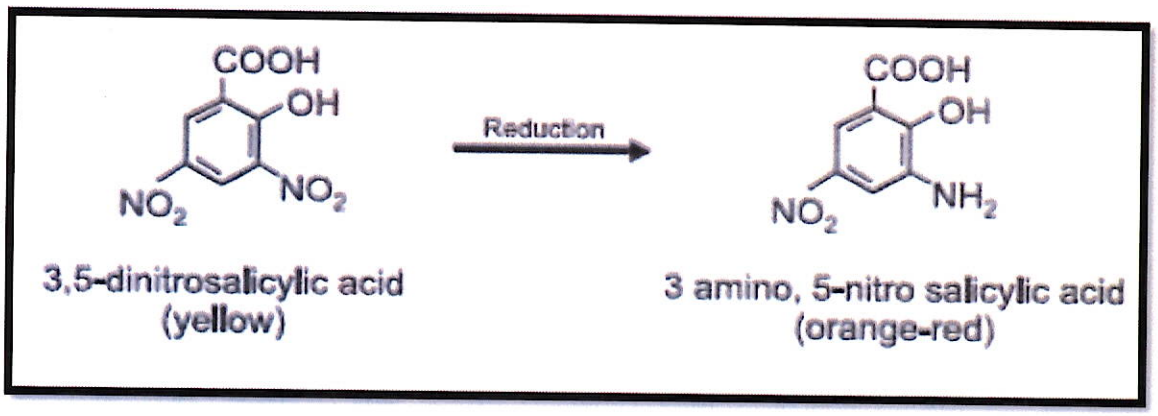
Observe the colour change of slant.

3. DETERMINATION OF CELLULASE ACTIVITY BY DNSA METHOD:

Bacterial colonies showing zones of hydrolysis were selected to determine cellulase activity. Selected bacterial colonies were inoculated in the CMC broth and incubated at room temperature for 24 - 48 hrs. After incubation, liquid broth is centrifuged at 10,000 rpm for 15 minutes. Supernatant is collected and used as a sample to determine the amount of reducing sugars released by the DNSA Method.

Principle of DNSA Method:

In biochemistry , 3, 5-Dinitrosalicylic acid (DNSA) method is widely used to estimate the amount of reducing sugars by detecting the presence of its free carbonyl group (C=O). Aldehyde and ketone, two functional groups found in sugars, are oxidized. During this process, under alkaline conditions, DNSA is reduced to 3- amino 5-nitrosalicylic acid (ANSA), which is reddish brown in color and this color intensity can be measured colorimetrically at 540 nm.



Img source: biocyclopedia.com

Figure 1: Reaction for DNSA

Requirements:

Sample	Crude enzyme extract
Media used	CarboxyMethyl Cellulose (CMC) broth
Standard used	1 mg/ml Glucose solution
Diluent used	Distilled water
Reagents used	3,5 -Dinitrosalicylic acid (DNSA)
Glasswares used	Sterile bumper tubes
	Sterile 5 ml pipettes
	Sterile eppendorf tubes
	Clean and dry test tubes
	Clean and dry 1 ml pipettes
	Clean and dry 10 ml pipettes
Instruments used	Cooling Centrifuge set at 10, 000 rpm
	Colorimeter set at 540 nm
Miscellaneous	Nichrome loop
	Tripod stand
	Cotton
	Disinfectant

Table 12: Requirement table for determination of cellulase activity by DNSA method.

Protocol:

For standard:

Make standard concentrations of glucose up to 1 mg/ml by adding standard glucose solution and diluent.

Add 1 ml of DNSA reagent in all test tubes and make a total volume of 2 ml.

Mix and keep all the test tubes in the boiling water bath for 15 minutes.

Cool and add 8 ml of distilled water and take O.D at 540 nm.

For unknown:

Take 1 ml of unknown sample in a test tube.

Add 1 ml of DNSA reagent in all test tubes and make a total volume of 2 ml.

Mix and keep all the test tubes in the boiling water bath for 15 minutes.

Cool and add 8 ml of distilled water and take O.D at 540 nm.

4. PROCESS OPTIMIZATION FOR CELLULOSE DEGRADING BACTERIA:

1. pH : CarboxyMethyl Cellulose (CMC) broth of different pH range 7, 8, 9, 10 ,and 11 is inoculated with 18-24 hrs old culture suspension of bacterial isolate and the broth is incubated at 37 ^{0}C for 24 -48 hrs . After incubation, the broth is centrifuged at 10,000 rpm for 20 minutes and the culture supernatant is used as enzyme source to determine its enzyme activity by the DNSA method .

2. Temperature : CarboxyMethyl Cellulose (CMC) broth with pH 7 is inoculated with 18-24 hrs old culture suspension of bacterial isolate and the broth is incubated at different temperatures such as 25 ^{0}C , 37 ^{0}C and 50 ^{0}C for 24 -48 hrs. After incubation, the broth is centrifuged at 10,000 rpm for 20 minutes and the culture supernatant is used as enzyme source to determine its enzyme activity by the DNSA method.

Requirements:
For optimization:

1	Culture	18 -24 hrs old culture suspension of cellulase producing bacterial isolate
2	Media	1. CarboxyMethyl Cellulose (CMC) Broth
		2. CarboxyMethyl Cellulose (CMC) Broth with different pH range from 7-11.
3	Glasswares	1. Sterile bumper tubes
		2. Sterile 10 ml pipettes
4	Instrument	1. Weighing balance
		2. pH meter
		3. Hot air oven set at 50^{0}C
		4. Incubator set at 37^{0}C

		5. Autoclave set at 121^{0}C
5	Miscellaneous	1. Disinfectant
		2. Cotton
		3. Tripod stand

Table 13: Requirement table for optimization of cellulose degrading bacteria.

For DNSA method:

1	Sample	Crude enzyme extract
2	Reagents	3,5-Dinitrosalicylic acid solution
3	Glasswares	1. Clean and dry test tubes
		2. 1 ml pipettes - 2
		3. 10 ml pipettes -2
4	Instruments	1. Boiling water bath
		2. Colorimeter set at 540 nm
		3. Centrifuge machine
5	Miscellaneous	1. Distilled water
		2. Test Tube stand
		3. Eppendorf tubes

Table 14: Requirement table for DNSA method.

INSTRUMENTS USED:

1. Weighing balance:

Img source: Contech Instrument Ltd.

Figure 2: Weighing balance

Principle: The analytical balance operates on the principle of electromagnetic compensation. Instead of directly measuring the object's weight, it uses an electromagnet to measure forces acting downward on a balanced plate.

A force is generated against the object's mass by supplying electrical current to the electromagnetic servo motor below the balance plate. The current necessary for this force to be generated is exactly proportional to the mass of the object, if the device is properly calibrated. The measured weights are then displayed on the screen. A null detector, which consists of a light source and a sensor, is used in many balances. It indicates that the weight and electromagnetic force are equal.

2. Autoclave:

Img source: Labindia instruments

Figure 3: Autoclave

Principle: In order to obtain a higher temperature and properly sterilize the material inside the chamber, autoclaves use steam under pressure to achieve moist heat sterilization. Water typically boils at 100°C under normal atmospheric pressure (760 mm of Hg). However, with higher pressure, the boiling point of the water increases. Heat can penetrate materials more rapidly at high pressures, and moisture in steam causes protein coagulation that leads to an irreversible loss of function and activity for microbes.

Autoclaves use this principle, where water boils at 121°C under a pressure of 15 psi or 775 mm of Hg. By releasing latent heat, the steam generated in the autoclave kills the microbes that come into contact with it. The condensed liquid ensures effective moisture-based microbial elimination. Once sterilization has been completed, the pressure inside the chamber is released by a whistle and returned to an ambient level. For a period following the procedure, the components in the chamber remain hot for some time.

3. Ph meter:

Img source:Alibaba.com

Figure 4: pH meter

Principle: A pH meter operates by exchanging ions between the sample solution and the inner solution (pH 7 buffer) of the glass electrode through a glass membrane. pH probes are composed of the sensor electrode and a reference electrode. The sensor electrode bulb, made of porous glass membrane coated with metal salts and silica, is filled with a pH 7 buffer, while the reference electrode is filled with potassium chloride solution.

Hydrogen ions are replaced by metal ions around the sensor electrode bulb when the pH probe is immersed in a sample solution. At the same time, some metal ions from the sensor electrode are transferred to the sample solution. The reference electrode, with its constant potential, remains insensitive to pH fluctuations. The potential difference between the electrodes produces an electric current which is captured by a silver wire, hydrogen ion activity. The pH meter will translate the electrical voltage into a pH value by comparing it to reference electrodes.

A rise in acidity of the solution leads to a greater concentration of hydrogen ions, which will lead to an increase in voltage. Consequently, the pH meter displays a decrease in pH readings. Conversely, an increase in alkalinity or the concentration of hydroxyl ions will decrease the voltage and cause pH readings to be increased on a pH meter.

4. Cooling centrifuge:

Img source: industrybuying.com

Figure 5: Cooling centrifuge

Principle: The centrifuge is operated on the basis of centrifugation technique in which particles are sedimented within the container, for example a test tube or bucket, using angular motion. This process enables particles of various sizes and shapes to be separated.

The particle experiences a centrifugal force in the centre of rotation when an angular velocity is applied. With a centrifuge, the centripetal force is generated by an upthrust of water media due to electrostatic repulsion resulting from charged particles present on sample particle surfaces. A pseudo force, known as Centrifugal Force, acts in the opposite direction from the center to counteract inward centripetal forces. The particles begin to move toward the center due to their inertia, which results in a separation process. The rate of sedimentation may vary between particles, particles with higher rates settle more quickly at the base which allows the separation of particles to take place.

5. Colorimeter

Img source: moglix

Figure 6: Colorimeter

Principle: The colorimeter works on the basis of Beer-Lambert's law, which states that the absorption of light passing through a medium is directly proportional to the concentration of the medium.

A beam of light with a specific wavelength is directed through a solution using lenses in the colorimeter. The colored light is then evaluated in comparison with the standard. The absorbance or percent of transmission is calculated by the microprocessor.

If the concentration of the solution is high, more light is absorbed and can be measured by comparing the amount of light before or after it passes through the solution. A number of solutions with known concentrations will be prepared and tested in order to determine the concentration of an unknown sample. A calibration curve will be created by plotting the concentrations and corresponding absorbance values. By comparing the absorbance value to the calibration curve, the concentration of the unknown sample can be determined.

6. Hot air oven :

Img source: Instech Systems

Figure 7: Hot air oven

Principle : The hot air oven operates based on the principles of convection, conduction, and radiation to achieve dry air sterilization. The temperature of the air will rise due to heating elements in the oven chamber. To ensure that all surfaces of samples are covered with warm and damp air, fans circulate the heated air in an evenly distributed manner. his exposure leads to the heating of the outer surfaces of items, and through conduction, the heat is transferred towards the center of the items. Microorganisms are affected by temperature, including water evaporation inside them that causes oxidative damage to cell components, protein denaturation, toxic effects of elevated electrolyte levels and ultimately the death of microorganisms.

7. Boiling Water Bath:

Img source: TradeIndia

Figure 8: Boiling water bath

Principle: The water temperature is converted into a resistance value by the sensor, which is then amplified and compared with an integrated amplifier. This also creates a control signal, which is capable of regulating the average heat output of an electric heater and maintaining water temperature constantly.

OBSERVATIONS

1. SCREENING AND ISOLATION OF CELLULOSE DEGRADING BACTERIA:

Soil characteristics:

1	Colour	Blackish grey
2	Texture	Clayey texture
3	Structure	Aggregated
4	pH	7.0
5	Temperature	25^0C

Table 15: Organoleptic properties of soil.

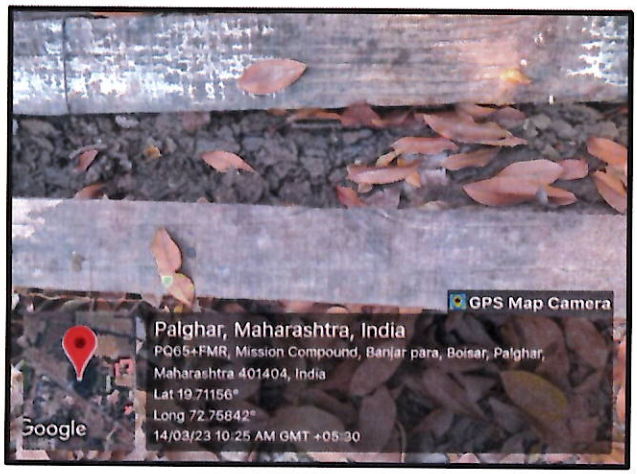


Figure 9: Location of the soil sample collection .

PLATE BEFORE STAINING	PLATE AFTER STAINING WITH CONGO RED

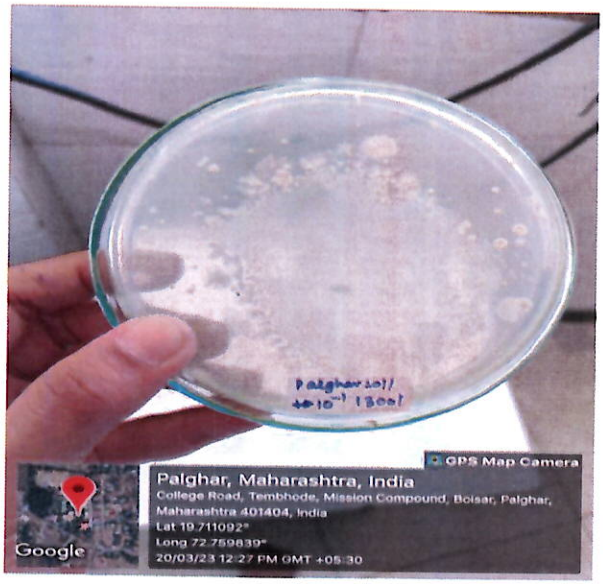
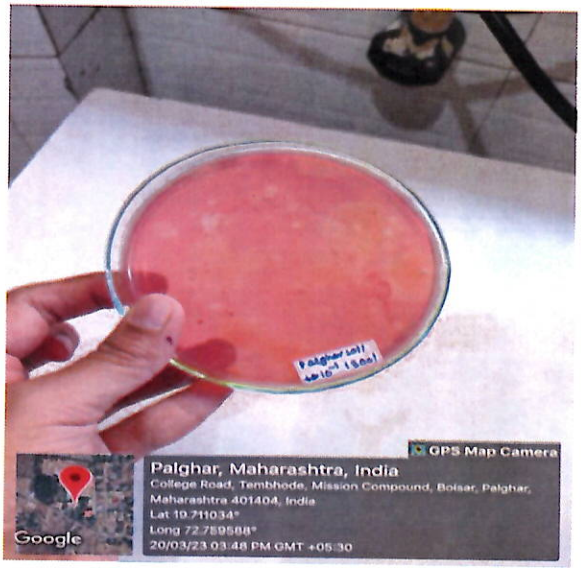
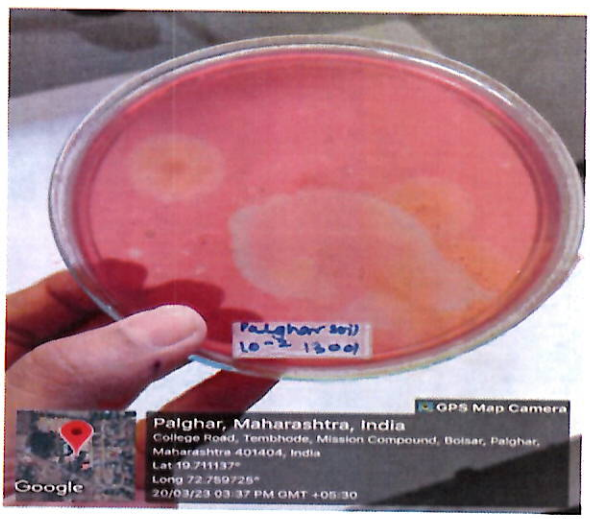

	

Figure 10: Spread plate of CMC agar containing 10 $^{-1}$ diluted soil sample.	*Figure 11: 10 $^{-1}$ dilution plate showing zone of hydrolysis after staining with 0.1% congo red solution.*

	
Figure 12: Spread plate of CMC agar containing 10 $^{-2}$ diluted soil sample.	*Figure 13: 10 $^{-2}$ dilution plate showing zone of hydrolysis after staining with 0.1% congo red solution.*

2. MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF CELLULOSE DEGRADING BACTERIA:

- **Morphological characteristic :**

Sr.no	Morphology	Observation
1	Size	1-2 mm
2	Shape	Circular
3	Colour	Off white
4	Margin	Regular
5	Elevation	Convex
6	Consistency	Mucoidal
7	Opacity	Opaque
8	Gram nature	Gram positive cocci

Table 16: Morphological characteristics of cellulose degrading bacteria.

- **Gram staining :**

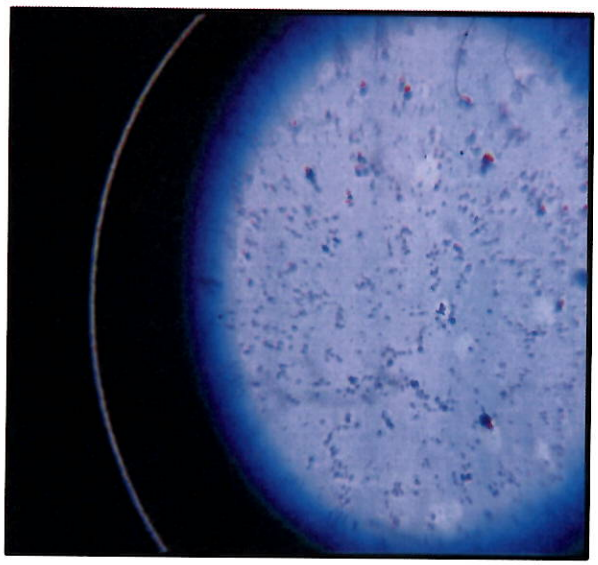


Figure 14: Gram positive cocci shaped cellulose degrading bacteria under 100X lens of microscope.

- **Biochemical Characteristics :**

Observation table:

1% Glucose		1% Sucrose		1% Mannitol		Indole	MR	VP	Citrate utilization	Gelatin hydrolysis	Starch hydrolysis	TSI
Acid	Gas	Acid	Gas	Acid	Gas							
+	+	+	+	+	+	-	+	-	+	-	+	Red slant & yellow butt

Key: (-) – positive test
(+) – negative test

Table 17: Observation table for biochemical characteristics.

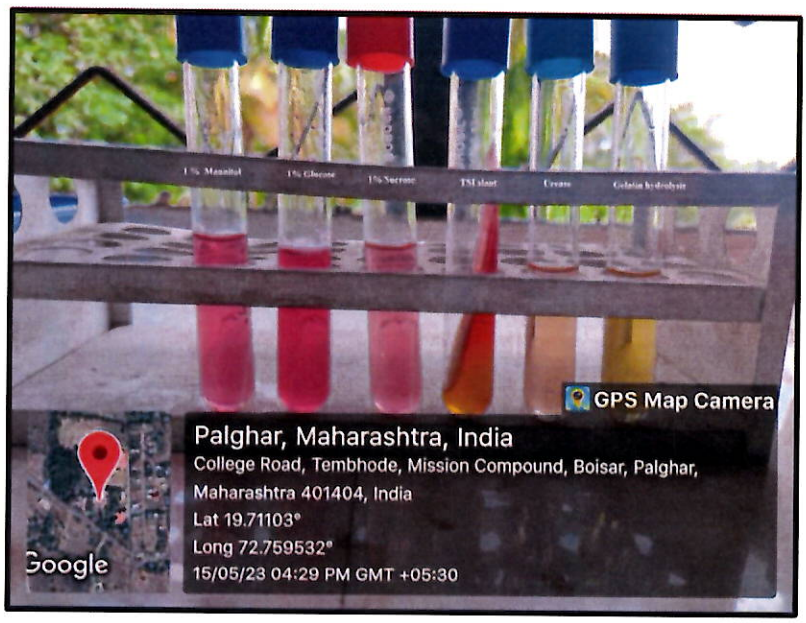


Figure 15: Observation for biochemical characteristics

Figure 16: Observation for the IMViC test.

- **Starch hydrolysis :**

Figure 17: Plate showing zone of hydrolysis on starch agar.

3. DETERMINATION OF ACTIVITY OF CELLULOSE DEGRADING BACTERIA BY DNSA METHOD:

Observation table:

Tube no .	Concentration of standard glucose (mg/ml)	Volume of standard glucose (ml)	Volume of diluent (ml)	Volume of DNSA reagent (ml)	Total volume (ml)		O.D at 540 nm
1	Blank	-	1.0	1.0	2.0	Mix and keep all the test tubes in the boiling water bath for 15 minutes .Cool and add 8 ml of distilled water and take O.D at 540 nm	0.0
2	0.1	0.1	0.9	1.0	2.0		0.04
3	0.2	0.2	0.8	1.0	2.0		0.09
4	0.3	0.3	0.7	1.0	2.0		0.16
5	0.4	0.4	0.6	1.0	2.0		0.21
6	0.5	0.5	0.5	1.0	2.0		0.33
7	0.6	0.6	0.4	1.0	2.0		0.35
8	0.7	0.7	0.3	1.0	2.0		0.42
9	0.8	0.8	0.2	1.0	2.0		0.45
10	0.9	0.9	0.1	1.0	2.0		0.52
11	1.0	1.0	-	1.0	2.0		0.53
12	10^{-1} I1	1 ml of crude enzyme extract		1.0	2.0		0.20
13	10^{-1} I2	1 ml of crude enzyme extract		1.0	2.0		0.09
14	10^{-1} I3	1 ml of crude enzyme extract		1.0	2.0		0.03

15	10^{-1} I4	1 ml of crude enzyme extract	1.0	2.0		0.04
16	10^{-2} I1	1 ml of crude enzyme extract	1.0	2.0		0.08
17	10^{-2} I2	1 ml of crude enzyme extract	1.0	2.0		0.03

Table 18: Observation table for determination of cellulase activity by DNSA method.

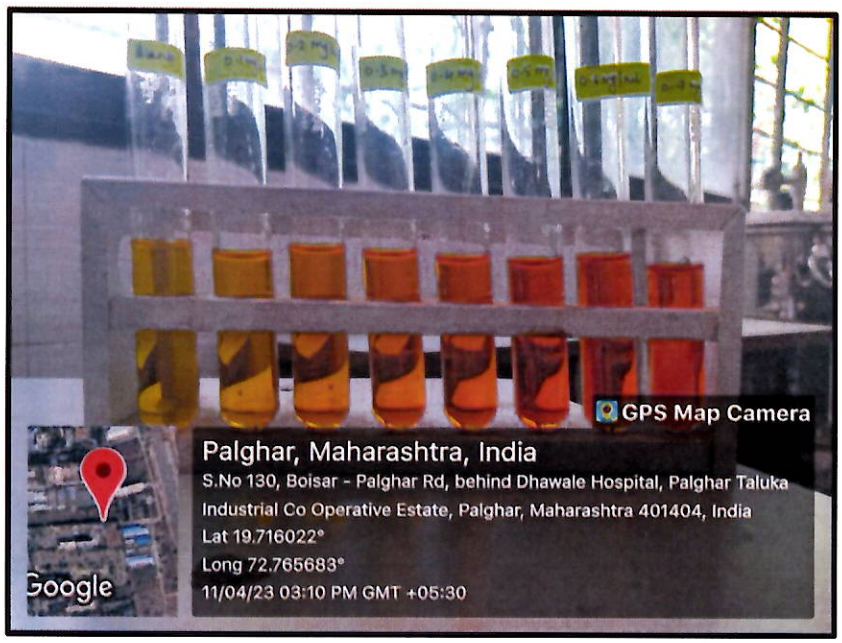


Figure 18a: Tubes containing Standard Glucose concentration for DNSA method.

Figure 18b: Tubes containing Standard Glucose concentration for DNSA method.

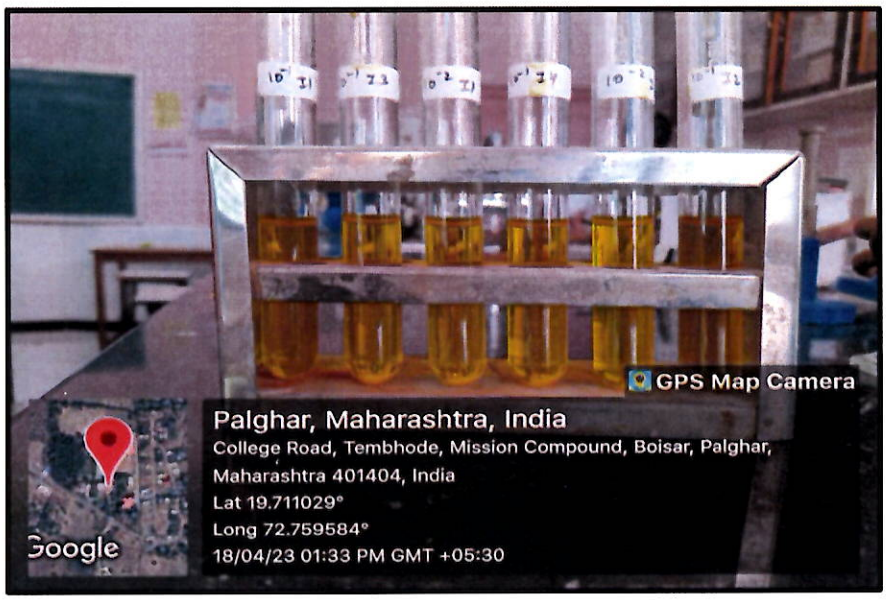


Figure 19: Tubes containing unknown samples for the DNSA method.

4. PROCESS OPTIMIZATION OF CELLULOSE DEGRADING BACTERIA:

Observation table:

Tube no.	Tubes	Volume of crude enzyme extract ml	Volume of DNSA reagent Ml	Total volume ml		O.D at 540 nm
1	25^0C	1.0	1.0	2.0	Mix and keep all the test tubes in the boiling water bath for 15 minutes .Cool and add 8 ml of distilled water and take O.D at 540 nm	0.0
2	37^0C	1.0	1.0	2.0		0.0
3	50^0C	1.0	1.0	2.0		0.0
4	pH 7	1.0	1.0	2.0		0.0
5	pH 8	1.0	1.0	2.0		0.0
6	pH 9	1.0	1.0	2.0		0.0
7	pH 10	1.0	1.0	2.0		0.0
8	pH 11	1.0	1.0	2.0		0.0

Table 19: observation table for determination of enzyme activity by DNSA method at 0 minutes of incubation.

Tube no.	Tubes	Volume of crude enzyme extract ml	Volume of DNSA reagent ml	Total volume ml		O.D at 540 nm
1	25^0C	1.0	1.0	2.0	Mix and keep all the test tubes in the boiling water bath for 15 minutes .Cool and add 8 ml of distilled water and take O.D at 540 nm	0.11
2	37^0C	1.0	1.0	2.0		0.03
3	50^0C	1.0	1.0	2.0		0.09
4	pH 7	1.0	1.0	2.0		0.03
5	pH 8	1.0	1.0	2.0		0.04
6	pH 9	1.0	1.0	2.0		0.01
7	pH 10	1.0	1.0	2.0		0.03
8	pH 11	1.0	1.0	2.0		0.00

Table 20: Observation table for determination of enzyme activity by DNSA method at 48 hrs of incubation.

Figure 20: Tubes containing CMC media after incubation at different pH and temperature.

Figure 21: Determination of enzyme activity by DNSA method after 0 minutes of incubation.

Figure 22: Determination of enzyme activity by DNSA method after 48 hrs of incubation.

RESULT AND DISCUSSIONS

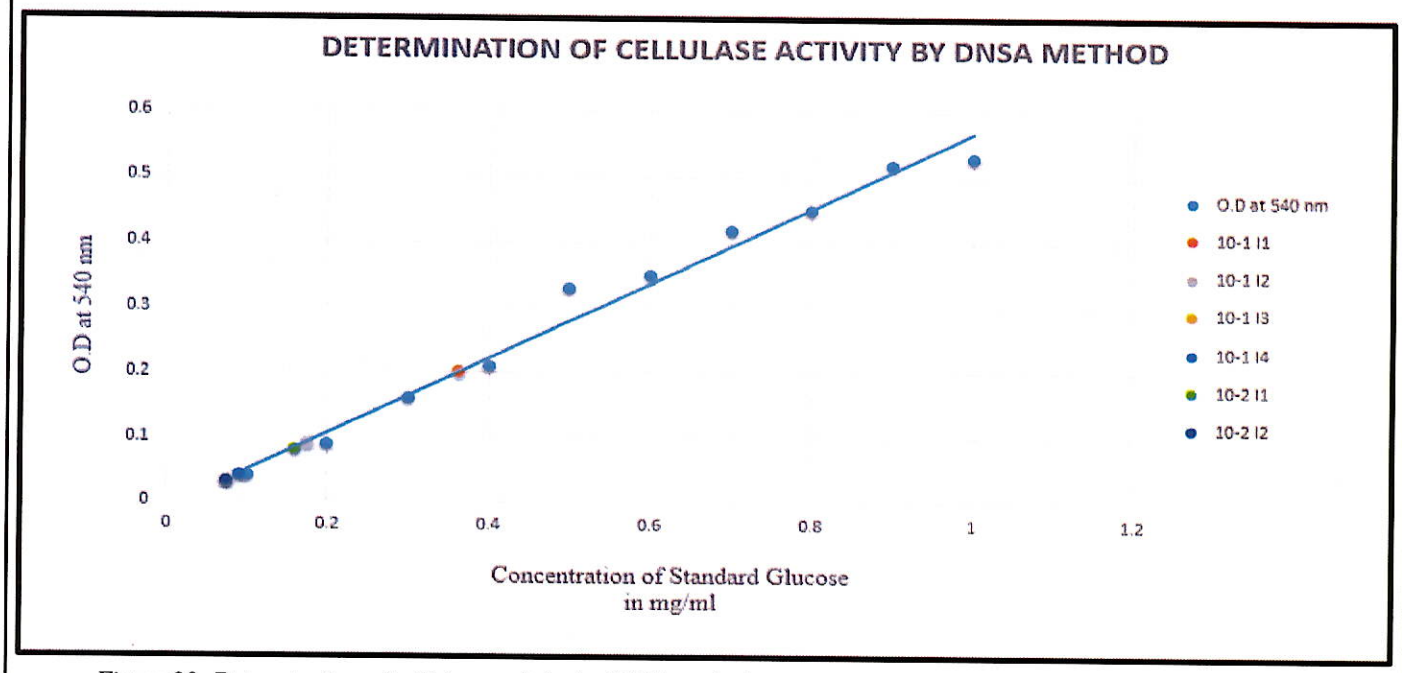


Figure 23: Determination of cellulase activity by DNSA method.

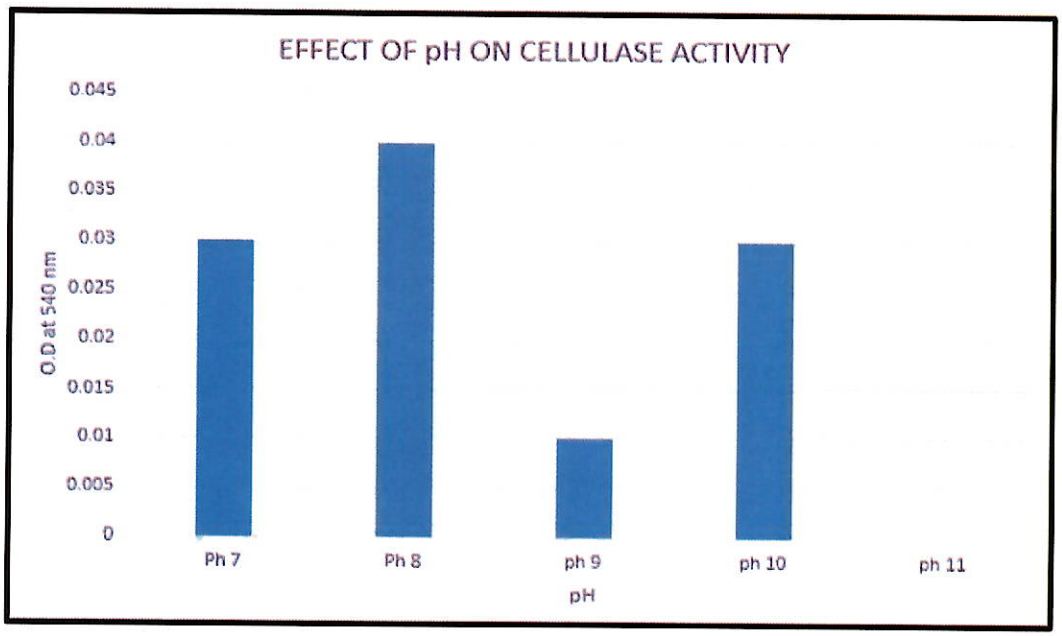


Figure 24: Graphical representation of effect of pH on cellulase activity

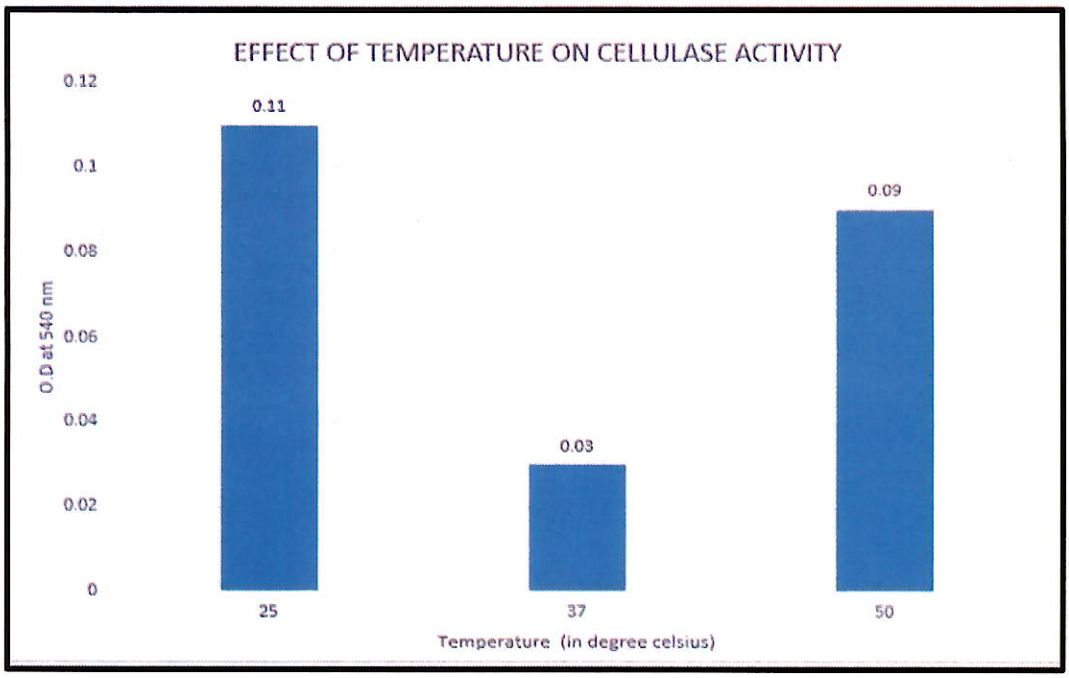


Figure 25: Graphical representation of effect of temperature on cellulase activity.

Cellulose degrading bacteria was isolated and screened from soil samples collected from the garden area of SDSM college, Palghar by a spread plate method on the Carboxymethyl cellulose medium. The clear zone of hydrolysis was observed around the colonies , when stained with 0.1 % Congo red solution which indicated the presence of cellulose degrading bacteria .

The bacterial isolate was then further characterized by studying its morphological and biochemical characteristics. The small, circular ,1-2 mm , off white mucoid colonies were observed on the plates when morphologically studied . Gram positive cocci shaped bacteria was observed under the oil immersion lens of a microscope. The bacterial isolate was found to be glucose , sucrose and mannitol fermenter along with the gas production, positive for Methyl red , Citrate , TSI and starch hydrolysis test but negative for Indole , Voges Proskauer and Gelatin hydrolysis where in the study carried out by Nandimath et.al in 2016 , they screened and isolated 3 cellulose degrading bacteria from soil and identified them as *Pseudomonas species* and *Bacillus species* which are gram negative and gram positive bacilli , respectively. Also in the study carried out by Sethi et.al in 2013, they screened and isolated 4 bacterial isolates from soil which are gram negative *Pseudomonas fluorescens, E.coli , Serratia marcescens* and gram positive *Bacillus subtilis* .

The maximum cellulase activity of isolated cellulose degrading bacteria was determined by estimating the amount of reducing sugars released during fermentation by the DNSA method and it was found to be 0.36 mg / ml .

Optimisation of cellulose degrading bacteria was carried out by considering process parameters like pH (7 - 11) and temperature (25°C , 37°C and 50°C). The maximum cellulase production was observed at 25°C at pH 8. The study carried out by Nandimath et.al in 2016, they found the maximum cellulase production at 30 °C at pH 5 by Pseudomonas sp.

CONCLUSION

The aim of this study was to screen, isolate, characterize and optimize the cellulose degrading bacteria from soil. The cellulose degrading bacteria was isolated and its characterization was carried out. The isolate was found to be Gram positive cocci shaped bacteria when morphologically and microscopically studied and found to be glucose , sucrose and mannitol fermenter along with gas production also positive for methyl red , citrate utilization, TSI and starch hydrolysis by biochemical characterization.

The maximum cellulase activity among isolates was determined by the DNSA method and the isolate with highest activity i.e., 0.36 mg/ml was further proceeded for process optimization. The parameters like pH and temperature were optimized. The maximum cellulase activity of cellulose degrading bacteria was found at 25^0C at pH 8.

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FUTURE PROSPECTS

- Use of cellulolytic enzymes has attracted many interests of researchers. As cellulases has applications in various fields such as food industries, detergent industries, textile industries, in bioethanol production, pharmaceutical industries, etc.
- It can also be used in industrial and agricultural waste management such as citrus peel waste, sawdust, paper pulp, industrial waste, municipal solid waste and paper mill sludge, etc.
- The crude enzyme extract can be purified by using ammonium sulfate precipitation, dialysis, and column chromatography.
- Also the isolated cellulolytic bacteria can be identified by using 16s rRNA sequencing technology.
- The enzyme purified can be further modified by recombinant DNA technology for high activity, yield and stability which can be used in various industrial and biotechnological applications.

APPENDICES

1. Carboxymethyl Cellulose Medium :

1	Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	0.1 g
2	Potassium chloride (KCl)	0.02 g
3	Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	0.1 g
4	Yeast extract	0.1 g
5	Carboxymethyl Cellulose (CMC)	1 g
6	Agar	1.5 g
7	Distilled water	100 ml
8	pH	7.0 -/+ 0.2

Table 21: Media composition of Carboxymethyl Cellulose Medium.

2. **0.1 % Congo red solution:** 0.05 g Congo red dye in 40 ml distilled water + 10 ml 95 % ethanol. Dilute in 1:1 ratio.

3. **Glucose phosphate broth:** 0.5 g glucose phosphate broth + 50 ml distilled water.

4. **Tryptone broth** :

1	Tryptone	0.5 g
2	NaCl	0.25 g
3	Distilled water	50 ml
4	pH	7.5 -/+ 0.2

Table 22: Media composition of tryptone broth.

5. Triple sugar iron (TSI) slant:

1	Beef extract	0.15 g
2	Yeast extract	0.15 g
3	Peptone	1 g
4	Glucose	0.05 g
5	Lactose	0.5 g
6	Sucrose	0.5 g
7	Ferrous sulfate or ferrous ammonium sulfate	0.01 g
8	NaCl	0.25 g
9	Sodium thiosulfate	0.015 g
10	Phenol red	0.001 g
11	Agar	0.65 g
12	Distilled water	50 ml
13	pH	7.3

Table 23: Media composition of TSI slant .

6. Simmon citrate slant:

1	Magnesium sulfate	0.01 g
2	Ammonium dihydrogen phosphate	0.05 g
3	K_2HPO_4	0.05 g
4	Sodium citrate	0.1 g
5	NaCl	0.25 g
6	Agar	1.5 g
7	Distilled water	50 ml

Table 24: Media composition of simmon citrate slant.

7. Starch agar:

1	Meat extract	0.15 g
2	Peptone	0.25 g
3	Starch	0.1 g
4	Agar	0.75 g
5	Distilled water	50 ml
6	pH	7.2

Table 25: Media composition of starch agar.

8. Nutrient Gelatin agar:

1	Gelatin	2.4 g
2	Beef extract	0.06 g
3	Peptone	0.1 g
4	Distilled water	20 ml
5	pH	6.8 -/+ 0.1

Table 26: Media composition of nutrient gelatin agar.