ISSN No. : 2454-1516
March 2019, Vol. 4 No.-1

Peer Reviewed Journal

Shodh Darpan

An International Research Journal

Published by
CHRIST COLLEGE
GEEDAM ROAD, JAGDALPUR, DIST.-BASTAR (C.G.) 494001, INDIA
Email : christcollegejagdalpur@gmail.com

March-2019, Vol.- 4 No. - 1, ISSN No. - 2454-1516

SHODH DARPAN

(A Half Yearly International Research Journal)

Website : http://christjagdalpur.in/journals.php
E-Mail ID : christcollegejagdalpur@gmail.com

MESSAGE

It is my privilege to present you with the Issue of *'Shodh Darpan'* whose mission is to provide an academic platform for discussing the issues of significant public interest on topics pertinent to a wider spectrum of content areas in various disciplines. *'Shodh Darpan'* tries to facilitate in-depth research and analysis of issues, concerns and challenges in various disciplines. The Sanskrit word *'Shodh Darpan'* means 'Search Mirror'. *'Shodh Darpan'* was started in 2015 and has stepped into Vol. 4 Issue No. 1 in March 2019. The journal targets scientists, experts, research scholars, policy makers, teachers, research centers, institutions, and centers of higher education. It is widely used by teachers, research scholars, and university students in various disciplines. All the papers submitted for publication in *'Shodh Darpan'* go through a double blind peer review process and plagiarism check. The journal accepts original research papers and reviews in various disciplines. This issue of *'Shodh Darpan'* focuses on the current issues and discourses in various disciplines. There is an eclectic mix of research papers and reviews that can enlighten the readers on various issues. We hope that our readers have an interesting reading and we also expect their continued support. I extend a great thanks and warm congratulations to all the contributors, Chief Editor and members on the editorial board for their valuable participation in different areas.

With greetings,
Fr. Shaiju P. K.
Co-ordinator, Research and Publication Cell
Christ College, Jagdalpur

DISCLAIMER

The information and opinions presented in the Journal reflect the views of the authors and not of the Journal or its Editorial Board or the Publishers/Editors. Publication does not constitute endorsement by the journal. Neither the Journal nor its Publishers/Editors/Editorial Board nor anyone else involved in creating, producing or delivering the Journal or the materials contained therein, assumes any liability or responsibility for the accuracy, completeness, or usefulness of any information provided in the journal, nor shall they be liable for any direct, indirect, incidental, special, consequential or punitive damages arising out of the use of information/material contained in the Journal. The journal, nor its Publishers/Editors/Editorial Board, nor any other part involved in the preparation of material contained in the Journal represents or warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such material. Readers are encouraged to confirm the information contained herein with other sources. The responsibility of the contents and the opinions expressed in this Journal is exclusively of the author(s) concerned.

- Editor

MESSAGE

St. Kuriakose Elias Chavara, our Founder, established a printing press in the state of Kerala to print the first Malayalam Daily Newspaper 'Deepika'. Printing many more things in the press, he opened to the world a new era of communication. Print media was and is still a powerful medium of communication.

'Shodh Darpan', a bi-annual publication, is a humble attempt of Christ College, Jagdalpur to provide a platform for scholars far and wide to share their knowledge and communicate their ideas, sharing in the explosion of knowledge of the present era. This research journal brings out the best papers of varied fields ensuring the validity of research results through thorough perusal.

As Christ College is embarking on a new phase of being recognized a research centre for scholars in the field of Chemistry and Computer Science, we are happy to bring out this research journal looking forward to open up new ventures in the area of research studies.

I congratulate Fr. Shaiju Payyappilly CMI, the Co-ordinator of Research and Publication Cell, Mr. Sushil Kumar Sahu, Chief-Editor, Co-Editors and members of Editorial Board of their constant effort in bringing out every issue of the Research Journal.

I do extend my sincere gratitude towards the Scholars who contribute by sending research papers on time which become the source and reason of publication of the journal. May God, the source of all wisdom, inspire minds to share the knowledge and contribute something to the world of knowledge.

Fr. Thomas V.V.
Principal
Christ College, Jagdalpur
Bastar Distt. (C.G.)

MEMBERS OF EDITORIAL BOARD

Guidelines to the Authors

1. Introduction of Journal

The journal **Shodh Darpan** is aimed at publishing original research in Computer Science, Physics, Chemistry, Social Work, Education, Microbiology, Biotechnology, Language & Literature, Social Sciences, Physical Sciences, Biological Sciences, Geography, Environmental Sciences & Applied Science. For apparatus and equipment used for experiments, manufacturers' names and model numbers are usually desirable.

2. Frequency of publication

Shodh Darpan is a Half Yearly Journal publishes in March and September months of the year.

3. Manuscript preparation

Manuscripts must be written in Clear, Concise, Grammatical English or Hindi. When preparing the manuscript, please remember -

A. The article must focus on *important new results*. Papers must be Concise, Lucid, and interestingthat will attract the reader to their work.

B. Title page:
   a) The title should be concise to describe the main importance of the work. Calibri 14 point fonts must be used for the title.
   b) The first name, middle name, and last name of each author along with their affiliating departments and institution are to be given.
   c) It should be clearly indicated who will handle correspondence at all stages of publication, also post-publication. The email address, phone no. and fax no. (if any) of the corresponding author must be given.
   d) A concise, single paragraph abstract of word limit from 50 to 200 words is to be given on the title page which will not contain any reference, figure or table.
   e) A minimum of 6 keywords are to be provided.

C. The paper in English must be typed in MS-Word (.doc) in Times New Roman 12 point with line spacing 1.5 with regular font style. For papers in Hindi author(s) must use 'KrutiDev 010' or 'Devlys 010' 15 point fonts with 1.5 line spacing. The heading of each section should be bold and sub-headings, if any, should be italicised.

D. The manuscript may contain sections like Introduction, literature review, Experimental/Methodology, Results and Discussion, Conclusion, Acknowledgement etc.
E. Figures, Tables, Charts and/or text that should be submitted with the manuscript so that it is clearly visible to the editors and reviewers.

3.1 Artwork

A. Uniform lettering and sizing of your original artwork are to be used.
B. Only 'Times New Roman' should be used in *Figures*.
C. The *Figures* are to be numbered according to their sequence in the text.
D. All symbols and abbreviations used are to be explained
E. Figures should be embedded in the paper in the correct position.
F. Resolution of the *Figures* must be as follows:
    a) For bitmapped line drawings, use a minimum of 1000 DPI and
    b) ForCombinations bitmapped line/half-tone (color or grayscale): a minimum of 600 x 600 DPI is required.
G. Tables*:* Tables should be numbered consecutively in accordance with their appearance in the text. The number of tables in the main article should be limited to include only the most important data. Tables that are not absolutely essential, as well as long and detailed tables should be placed in supplementary materials.

3.2. Reference Style

The style of referencing should be in MLA or APA

4. Mention of funding source

Financial support, if, any, should be acknowledged at the end of the article under the heading “Acknowledgement”.

5. Submission

Submission of the paper implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture in conferences/ seminars/symposia), that it is not under consideration for publication elsewhere, that its publication is approved by all authors, that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, without the written consent of **Shodh Darpan**.

The manuscript completed and prepared as per the guidelines stated above should be submitted to christcollegejagdalpur@gmail.com.

Note :

(1) A Declaration Form alongwith hardcopy and softcopy (in .doc format) of the paper has to be submitted by the author(s) at the time of submission of the paper.

(2) Plagiarizm of any kind will not be accepted. In such case the publisher has the right to reject the paper.

6. Copyright

By submitting an article to **Shodh Darpan** for publication author(s) accept the 'Publishing Agreement' and thereby transfer the copyright to the publisher of **Shodh Darpan**. However, the author(s) will retain certain rights such as to use for a patent, to use for the preparation of a lecture.

7. Subscription Fee

(i) Individual :

(a) Per Issue : Rs.400/-,

(b) Per Annum : Rs. 700/-

(ii) Institution :

(a) Per Issue :Rs. 600/-

(b) Per Annum : Rs. 1100/-

For subscription, please contact**:**

Fr. Shaiju P.K.,
Co-ordinator,
Research and Publication Cell,
Christ College,
Jagdalpur, Chhattisgarh, India
Email: christcollegejagdalpur@gmail.com

Note: The Author Guidelines are subject to modifications /upgradations from time to time.The Authors are therefore, requested to follow the latest version of the Author Guidelines.

FORMAT OF BONAFIDE DECLARATION FORM

I _____ designation _____ of _____ hereby delcare that the research work submitted, is an original piece of work done by me which has not been published anywhere else in best of my knowledge.

Name and Signature of the Author(s) with Date

CONTENTS

Mobile Phone Usage Pattern Among Adult Patients with Type 2 Diabetes Mellitus in a Sub-Center in Bengaluru Urban District – A Cross Sectional Study

Dr.Reni Philip[1]

(I) Abstract

Introduction: Diabetes education enables patients to be more involved in their care and helps in delaying complications and adverse health outcomes. Mobile phone penetration in rural India is high and provides us with an opportunity to use mobile phones in delivering diabetes education messages.

Objective: To study mobile phone usage pattern among adult patients with type 2 diabetes mellitus in Mugalur sub-Centre area.

Methodology: A list of all the known patients with type 2 diabetes mellitus was obtained from the health management information system records of the rural health and training Centre of the institution and a structured interview schedule was administered to the participants. A total of 250 out of a total of 311 diabetic patients present in the 10 villages under the sub-centre were included in the study.

Results: Around half of the diabetic patients included in the study (55.6%) owned a personal mobile phone, two thirds (62.4%) had access to a shared mobile phone at home, 44.4% had access to a smart phone in the household and 35.4% had internet connectivity on their phone. Predominant mobile phone usage pattern included making phone calls (54.4%), messaging services (44.8%), listening to music (17.6%), alarm (14.4%) and capturing photos / videos (12.8%). Very few participants reported using mobile phone for health-related purposes (12.8%) and usage of health-related apps (10%). A large proportion of the participants reported that they would like to receive reminders for clinic visits (54.0%) and set alarms as reminders to improve adherence to medication (53.6%) in diabetes.

Conclusion: Two thirds of the patients with known diabetes in rural areas have access to a mobile phone at the household level. Mobile phones have potential application to be used

[1] Post graduate student, Department of Community Health, St. John's Medical College, Bangalore

to send reminders for clinic visits and to improve adherence to medication among patients with diabetes.

(II) Key words

Mobile *phone, type 2 diabetes mellitus, rural.*

(III) Introduction

Diabetes mellitus, specially type 2 diabetes, is the most significant public health challenges of 21st century. More than 80% of diabetes death is in developing countries.

(1) The prevalence of diabetes for all age-groups worldwide was estimated to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030(WHO). Proper diabetes education can help the patients themselves to better manage their disease and successfully avoid complications of the same. However, health systems cannot control all the factors that affect a person's overall health, as doctors are not able to constantly monitor what their patients eat or whether they take their medications on time as prescribed by them.

(2) To prevent the above draw back and to attain that goal the medical professional can send SMS to remind the diabetic patients regarding the need for the adherence to medications. TELECOM REGULATORY AUTHORITY OF INDIA New Delhi, 24th April, 2018, published the data, total a telephone subscribers (in million) wireless are 1156.87, wireline are 22.97, total are 1179.83. The rural telephone subscribers are wireless 506.84, wireline 3.37, total 510.20 (data as on 28th February,2018).

(3) The core component is a mobile phone-based diabetes diary that is updated both by manual user input and automatically by wireless data transfer. It also provides personalized decision support by giving feedback on users' performance related to their personal health goals.

(4) The mobile phone based interventions helped overcome barriers such as stigma, loss of privacy, limitation of transportation, and problems associated with traditional interventions, and made participants feel cared for so that they were responsible for their treatment.

(5) These findings support the important role of mobile phone based interventions for promoting adherence to treatment.

(IV) Objectives

To study mobile phone usage pattern and acceptability of mobile phone to support the treatment among adult patients with type 2 diabetes mellitus in Mugalur sub-center.

(V) Methodology

Our study includes 311 diabetic population of Mugalur sub-center of Sarjapur district. Our sampling technique was universal sampling. Study design was cross sectional study. Our study population includes all diabetic who are above 18 years in our study area. All diabetic population of Mugalur sub-centre included in our study, and type 1 Diabetic population and the seriously ill person who are unable to answer the questions were excluded from the study. Data collection done after getting ethical clearance from Institutional Ethical Committee and informed written consent taken from patient. Type 2 diabetics patients are selected from the HMIS (Health Management Information System) of Mugalur. The total diabetic people of all 10 sub-center is about 311. The whole 311 patients are taken into account of my study. Administering a questionnaire comprising of social and demographic factors like age, socio-economic status using modified B.G.Prasad classification. Health status includes any chronic and acute health problems and duration of diabetes and drugs consuming for the diabetics and latest sugar values. Mobile phone and its usage pattern including hours on phone and using phone for health information and personal mobile phone. Knowledge and practices related to diabetes using details about how many times had gone for brisk walk, number of days anti diabetic medicine missed and number of times taken sweets and junk food in a week and knowledge about most common complications of diabetics included**.** Study period was July and August 2018.

(VI) Analysis

The data collected were entered in Microsoft Excel. The data were analyzed using percentages, mean and standard deviation. The various factors associated with mobile phone usage pattern were analyzed. Results depicted in tables and graphs. The data were analyzed using a standard statistical package (SPSS V. 16).

(VII) Results

Our study includes 311 diabetic population of Mugalur sub-center of Sarjapur district. Out of our sample population 155 (49.8%) were male and 156 (50.2%) were females and their mean age was 56. Around half of diabetic patients included in the study 170 (54.7%) owned a personal mobile phone. And 219 (70.4%) of diabetic people who had the access to share the mobile phone at home. 107(34%) who access the internet, 153 (49%) who don't possess internet access and 51(16%) of people who don't know about the internet facility. Out of 311 diabetic people of my study, number of mobile phones they possessed are 32% having one mobile phone, 57% is having two mobile phones, 9% having three mobile phones, 1% of having four mobile phones, 1% of them having five mobile phones in the houses. Chronic disease: 126(40.5%) had hypertension, 11(3.5%) had ischemic heart disease, 4 (1.3%) had osteoarthritis, 4(1.3%) had stroke of all 311 diabetic patients. Mean age of their duration of diabetics are 9.17 years. Acute disease: 91% of the people do not have any acute disease,

5% of them having cough and cold, 3% of them having fever, 1% of having any other diseases like headache, body pain etc. Women's health: Out of 156 women of our study group (total diabetic women population) 132(84.6%) do not have any women's health problems, 8(5.1%) have menstrual problems, 16 (10.3%) having white discharge per vagina.

305 people use mobile independently for phone calls, and 6 people use mobile phone for health with help. People who can use phone for messages are 121 independently and 140 people use mobile phone with help. 46 people listen to music independently and 57 people listen to music with help. 41 people uses alarm independently. 82 people uses mobile to keep alarm with help. 12 people uses mobile for social media independently and 19 people uses with help. 5 people uses mobile phone for video chat independently and 11 people uses with help. 4 persons uses mobile for browsing independently and 17 people uses with help. 1 person uses mobile phone for playing games independently 17 people uses with help. 6 people uses mobile phone for watching movies independently and 5 people uses with help. 16 people uses mobile phone for capture photo independently and 6 people uses with help.

Average time spent on browsing internet for health are 23% uses 45 minutes, 57% uses 30 minutes, 11% uses 15 minutes 9% of people uses 10 minutes. Problems faces while using mobile phone are difficulty in using phone are 123 (39.5%), bad internet connectivity 86 (27.7%), bad signal for 41 (13.2%) people and 80 (25.7%). Mobile phone reminder will help the people to reminder for clinical visits is 162(52.1%), to improve physical activity 106(34.1%), to improve dietary practices 70 (22.5%), reminder for lab tests 60(19.3%), to improve knowledge about the knowledge 65(20.9%). The types of reminder the patients would like to receive is phone calls 206 (66.2%), SMS 138 (44.4%), MMS 22(7%), WhatsApp messages 9 (4.2%), alarm 13 (4.2%). Facility for sharing the information if we send SMS to a person who own mobile phone is 296 (95.2%). According to patient knowledge of diabetic complications 259 (83.3%) have eye problems, 66 (21.2%) have kidney problems, 110(35.4%) have nerve problems,38(12.2%) have heart problems. According to the knowledge of patient components of diabetic foot care are, 203 (65%) says wearing comfortable footwear, 155 (49%) says wash and dry foot every day, 117 (37.6%) says treat injuries immediately, 8(2.6%) says cut toe nails straight, 15(4.8%) says examine foot every day. When we did chi-square test the knowledge of patients about component of diabetic foot care which came to be significant (<0.05) the person who uses mobile phone apps will have more knowledge about the need to keep the hygiene of nail by cutting it. The people who uses health related apps have more knowledge for brisk walk for thirty minutes than the people who are not using.

(VIII) Discussion

There are many trials using mobile phone as a tool for improving the adherence to diabetic medications around the world. Diabetes education was offered by some organizations via lectures, workshops, or pamphlets but not through mobile text messages. Very limited number of private hospitals had the possibility of sending their patients a text message, yet only in cases of appointment cancellations. The idea of SMS messages with educational content was regarded as promising, especially that it could aid hospitals meet the standard of patient education, one that is required by quality systems in Egypt(2). But there is not much study shows the effectiveness of SMS to the diabetic patients to improve their knowledge about diabetics and adherence to medications, especially for the good of rural people. Somy study can be a help to prove that the effectiveness of SMS to the diabetic patients to improve their knowledge.

Information and communication technologies (ICTs) have the power to influence the quality of life of peoples. They have been a key driver for many improvements in modern society including in health care, transport, education as well as public safety. (6) there are lot of research has been conducted into analysing how technology has been leveraged to enhance aspects such as productivity, efficiency and speed. (6) Diabetes does demand life-long medical care and self-management, it is difficult for the patients to motivated to take medicine regularly .Mobile technologies might help patients withstand the common reasons for dropping out. Recently, mobile technologies, such as smartphone applications (mHealth), have been shown to be effective in supporting the self-management of diabetes patients(7). A smartphone-based self-management support system provides a real-time advice on diet and lifestyle managements to maintain sugar levels . (7) The system significantly improved introduced to the patients by their physicians, it might help strengthen the physician–patient relationship, thereby enhancing the patient motivation that is especially necessary for continuous care by patients with mild glycaemic status. (7) Mobile phones can be a low cost solution to provide health education and increase adherence to treatment for people with chronic diseases like diabetes. (1) Poorly controlled diabetes leads to devastating complications at a significant cost to health systems. Text messaging is an ideal platform for the delivery of self-management interventions to patients with poorly controlled diabetes due to the ubiquity of mobile phones, and the ability of text messaging to reach people in their everyday lives when self-management of the condition is vital.(8)

The SMS intervention for diabetic patients really made a change in HbA1c level. It is seen more in developed country than in developing country.(9) Usage of tailored SMS reminders to increase adherence in treatment programs among sick individuals has suggested an interventional role for SMS in self-care management of Diabetes Mellitus (DM)(10)Findings

from a 6-month field study showed that good system usability and user acceptance. The diary also challenges patients to decide about how they can improve their situation, because it helps them a way to capture and analyze relevant personal information about their disease(4). Increasing availability and openness of embedded sensors in mobile devices will gradually helps in more accurate and adaptive apps that are aware of their context of use, e.g. the Ring Dimmer app(4). One study conducted in US shows that SMS messages send to a group of patients show improvements in their self-management behavior and foot checking and blood glucose monitoring and above all medication adherence. (2) Text-message reminders have been shown to be effective amplifiers of adherence in their medical needs. (8) Study demonstrates that good effective interventions like SMS for increasing activity in patients with pre-diabetes and diabetes remain as an urgent need and it will increase the compliance level to medication and other activity(8). Each person's behaviours play an important role in diabetes control including blood glucose monitoring, medication adherence, physical activity and healthy eating, and therefore diabetes self-management education and support is a fundamental part of diabetes care through media. There is a wide range of interventions designed to support people to self-manage their diabetes; from passive interventions (e.g. provision of information like SMS) to more active interventions (e.g. interventions to change behaviour or increase self-efficacy). (11)The idea of SMS messages with educational content was regarded aspromising, especially that it could aid hospitals meet the standard of patient education, one that is required by quality systems in Egypt(2). But there is not much study shows the effectiveness of SMS to the diabetic patients to improve their knowledge about diabetics and adherence to medications, especially for the good of rural people. What we found in our study was individuals who use their mobile phone for health go for more brisk walks and also cut toe nails straight. 57% of the study population was found to have 2 phones in their household. 98% of the diabetics use mobiles independently for phone calls, 45% use messages on their phones with help, 10% of the individuals use health related apps, 11.3% of the individuals browse the internet for health-related queries, 39.5% of the individuals found it difficult to use their mobile phones.

When questioned about how mobile phones will be of help it was found that reminders for clinic visits will be of most help and when asked about how they would like the reminders we found that Phone calls will be of most help. Individuals who use their mobile phone for health, health related apps and also browse the internet for health-related queries were found to consume lesser junk food when compared to those who do not use their phones for health. Individuals who use health related apps were found to have a better knowledge about heart problems as a complication of diabetes. Those who get health related information on their phones had a better knowledge about nerve problems as a

complication of diabetes. Those who share and receive health related information on mobile phones were found to inspect their feet every day with a mirror and also knew to wash and dry their feet every day. On the whole having a better knowledge about diabetic foot-care.

(IX) Conclusion

Two thirds of the patients with known diabetes in rural areas have access to a mobile phone at the household level. Mobile phones have potential application to be used to send reminders for clinic visits and to improve adherence to medication among patients with diabetes.

(X) Reference

1. Islam SMS, Lechner A, Ferrari U, Froeschl G, Alam DS, Holle R, et al. Mobile phone intervention for increasing adherence to treatment for type 2 diabetes in an urban area of Bangladesh: Protocol for a randomized controlled trial. BMC Health Serv Res. 2014;14(1):1–9.
2. Abaza H, Marschollek M. SMS education for the promotion of diabetes self-management in low & middle income countries: A pilot randomized controlled trial in Egypt. BMC Public Health. 2017;17(1):1–19.
3. TELECOM REGULATORY AUTHORITY OF INDIA 20 Glorious Years. 1997 [cited 2018 Jul 15]; Available from: www.trai.gov.in
4. Chomutare T, Sc M, Tatara N, Sc M, Hartvigsen G, Ph D. Mobile Health Applications to Assist Patients with Diabetes: Lessons Learned and Design Implications. 2012;6(5):1197–206.
5. Chen S, Pan Y, Qu Y, Liu Z, Yao L, Xiang L. Mobile phone based interventions for promoting adherence to tuberculosis treatment: a systematic review and meta-analysis. Lancet [Internet]. 2015 Nov 13 [cited 2018 Sep 2];386:S29. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0140673615008673
6. Makgopela R, Van Belle J. The Impact of Mobile Phones on Quality of Life of the Elderly. 8th Int Dev Informatics Assoc Conf ICTs Incl communities Dev Soc (IDIA2014), Port Elizab South Africa. 2014;(2014):223–40.
7. Yamaguchi S, Waki K, Tomizawa N, Waki H, Nannya Y, Nangaku M, et al. Previous dropout from diabetic care as a predictor of patients' willingness to use mobile applications for self-management: A cross-sectional study. J Diabetes Investig. 2017;8(4):542–9.
8. Polgreen LA, Anthony C, Carr L, Simmering JE, Evans NJ, Foster ED, et al. The effect of automated text messaging and goal setting on pedometer adherence and physical activity in patients with diabetes: A randomized controlled trial. PLoS One. 2018;13(5):1–12.
9. Defronzo RA, Simonson DC. Oral sulfonylurea agents suppress hepatic glucose

production in non-insulin-dependent diabetic individuals. Diabetes Care. 1984;7(SUPPL. 1):72–80.
10. Riaz T, Riaz H, Hussain SA, Kherani D. SMS reminders- future in self-care management of diabetes mellitus? Diabetol Metab Syndr. 2012;4(1):3–4.
11. Dobson R, Whittaker R, Pfaeffli Dale L, Maddison R. The effectiveness of text message-based self-management interventions for poorly-controlled diabetes: A systematic review. Digit Heal [Internet]. 2017;3:205520761774031. Available from: http://journals.sagepub.com/doi/10.1177/2055207617740315

(XI) Tables and Graphs

Table:1 Sociodemographic details of study population:(n=311)

Sex	Male	155(49.8%)
	Female	156(50.2%)
Age (mean ± SD) years	56.10± 12.13 years	
Education	No formal education	95(30.55%)
	Primary school	93 (29.90%)
	Middle school	61(19.61%)
	High school	47(15%)
	Pre-university	10 (3.21%)
	Graduate	5 (1.60%)
Occupation	House wife	134 (43%)
	Unemployed	67 (22%)
	Business	58 (19%)
	Agricultural land owner	24 (8%)
	Daily wage earner	19 (6%)
	Salaried	6 (2%)
	Business	2 (0.64%)
	Retired	1 (0.32%)
SES (socio economic status) B.G.Prasad classification:	Upper Class	21 (6.75%
	Upper Middle Class	54(17.36%)
	Middle class	97 (31.19%)
	Lower middle class	96 (30.87%)
	Lower class	43 (13.87%)
Religion	Hindu	299(96.1%)
	Muslim	11(3.5 %)
	Christian	1(0.3%)
Language	Kannada	244 (78.5%)
	Telugu	213 (68.5%)
	English	13 (4.2%)
	Others	19 (6.1%)
Total		311(100%)

Graph 1 : Mobile Phone Usage Pattern

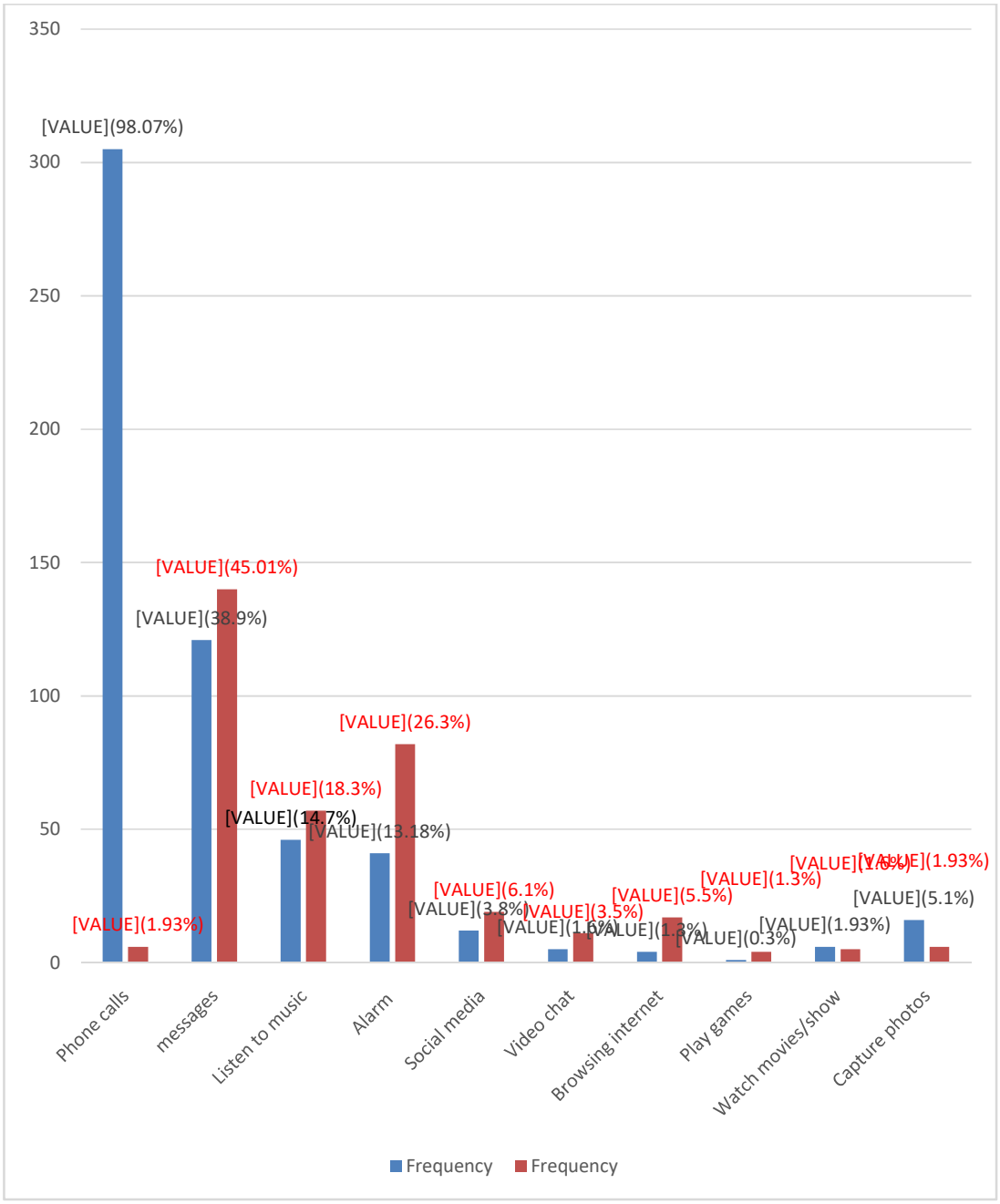


Table 2 : Average usage of Mobile Phones for different purposes

Questions	Number	Percentage
People using mobile phone for health	38	(12.2%)
People having health related apps	31	(10%)
People using mobile to share health related information(forwards)	64	(20.6%)
People receiving health related information(forwards) on mobile	85	(27.3%)
People who browse internet for health-related queries :	35(N)	(11.3%)
1) Immediate answers	16	(45.7%)
2) Good information	12	(34.3%)
3) Don't want to ask others	3	(8.6%)
4) More convenient	8	(22.9%)
People who do not browse internet for health-related queries:	276 (N)	(88.7%)
1) Not aware of sites	66	(23.9%)
2) Don't know how to use	196	(71%)
3) Don't think its useful	30	(10.9%)

Graph 2: Mobile Phone Reminder Helpful for Health

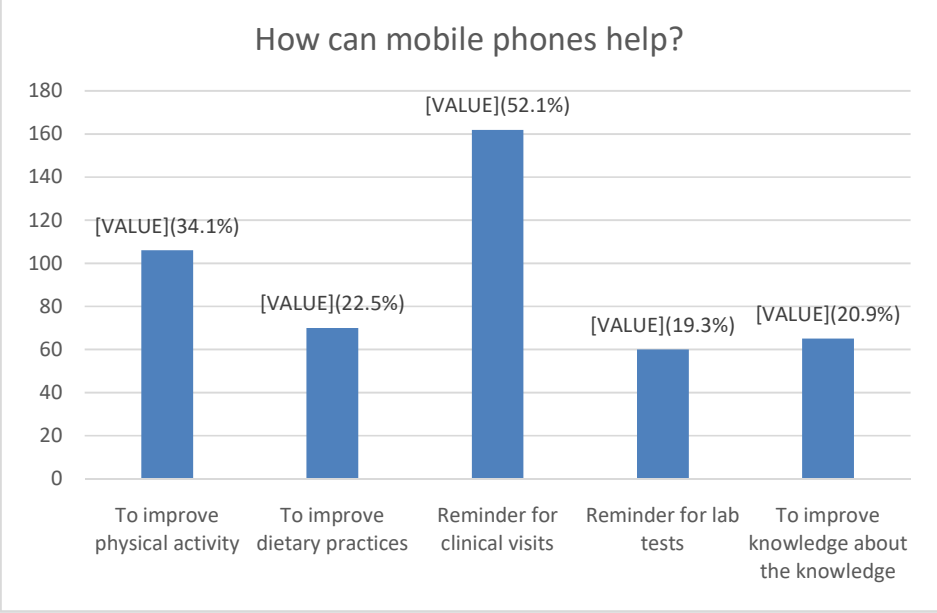


Table 3 : Knowledge about Brisk Walk and Mobile Phone Usage Pattern

VARIABLE		IN THE LAST 7 DAYS HOW MANY DAYS PATIENTS GO FOR A BRISK WALK FOR AT LEAST 30 MINS?		p VALUE
		GOOD	POOR	
DO YOU USE MOBILE PHONE FOR HEALTH?	YES	31(81.6%)	7(18.4%)	0
	NO	139(50.9%)	134(49.1%)	
DO YOU HAVE HEALTH RELATED APPS ON PHONE?	YES	26(83.9%)	5(16.1%)	0.001
	NO	144(51.4%)	136(48.6%)	
DO YOU BROWSE INTERNET FOR HEALTH RELATED QUERIES?	YES	29(82.9%)	6(17.1%)	0
	NO	141(51.1%)	135(48.9%)	
DO YOU SHARE HEALTH RELATED INFORMATION ON PHONE?	YES	46(71.9%)	18(28.1%)	0.002
	NO	124(50.2%)	123(49.8%)	
DO YOU GET HEALTH RELATED INFORMATION ON PHONE?	YES	56(65.9%)	29(34.1%)	0.015
	NO	114(50.4%)	112(49.6%)	

Table 4 : Knowledge About Diabetic Foot Care

VARIABLE		KNOWLEDGE ON DIABETIC FOOT CARE: CUT TOR NAILS STARIGHT		p VALUE
		GOOD	POOR	
DO YOU USE MOBILE PHONE FOR HEALTH?	YES	5(13.2%)	33(86.8%)	0(0.001*)
	NO	3(1.1%)	270(98.9%)	
DO YOU HAVE HEALTH RELATED APPS ON PHONE?	YES	6(19.4%)	25(80.6%)	0(0*)
	NO	2(0.7%)	278(99.3%)	
DO YOU BROWSE INTERNET FOR HEALTH RELATED QUERIES?	YES	5(14.3%)	30(85.7%)	0(0.001*)
	NO	3(1.1%)	273(98.9%)	
DO YOU SHARE HEALTH RELATED INFORMATION ON PHONE?	YES	7(10.9%)	57(89.1%)	0(0*)
	NO	1(0.4%)	246(99.6%)	
DO YOU GET HEALTH RELATED INFORMATION ON PHONE?	YES	8(9.4%)	77(90.6%)	0(0*)
	NO	0(0%)	226(100%)	

Secondary Metabolite Production Through Plant Tissue Culture Technique

Akanksha Mahna Anand[1]

(I) Abstract

Catharanthus roseus is an important medicinal plant. Generally, various pieces of the plant is utilized in the medications of different maladies like diabetes, menstrual controllers, hypertension, malignant growth and so forth., in quantities of nations like Australia, Brazil, China, England, India, Pakistan and so forth. Additionally, in excess of 130 alkaloids have been secluded from various pieces of the plant which produces anticancer dimeric alkaloids, for example, vinblastine and vincristine in convergences of 0.0004 to 0.0003% dry loads in leaves. The low yields of the antileukemic alkaloids in the plant, combined with their high market value have encouraged intense research for finding alternative methods for the large production of vinblastine and vincristine alkaloids, through tissue culture.

Sustaining forerunner has a job on the auxiliary metabolite upgrade in vitro culture. The impact of L-tryptophan on catharanthine generation was considered in callus culture of Catharanthus roseus. Callus culture of C.roseus was refined on the Murashige and Skoog (MS) medium which was enhanced with various convergence of hormones like auxin and cytokinins Combination of auxins and cytokinins were found to be better for leaf callus growth. The highest value of mass were achieved with MS medium containing 0.50 mg/l of 2, 4-D and 1.0 mg/l of BAP compared with other combination and this callus were again sub cultured on MS medium containing different concentration of L-tryptophan i.e., 150, 175, 200 mg/l.

Quantitative analyses were conducted using thin layer chromatography (TLC) and no alkaloids were present in the callus culture of *Catharanthus roseus.*

(II) Key words

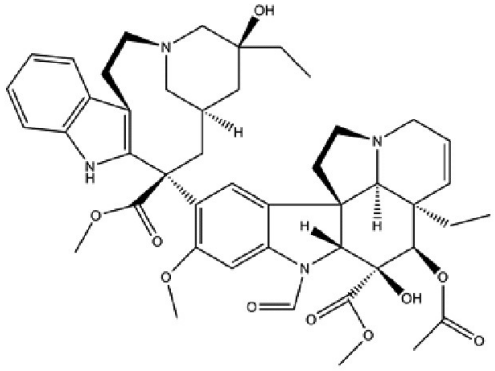
TDC – Tryptophan decarboxylase, **STR** – Strictosidine synthase, **SGD** – Strictosidine glucosidase, **T16H**– Tabersonine 16-hydroxylase, **NMT** - 16-methoxy-2, 3-dihydro-3-hydroxy tabersonine*N*-methyltransferase, **D4H** - Vindoline-4-hydroxylase, **DAT**- Deacetylvindoline*O*-acetyltransferase

[1] Asst. Prof., Christ College, Jagdalpur, Bastar Distt. (C.G.), India

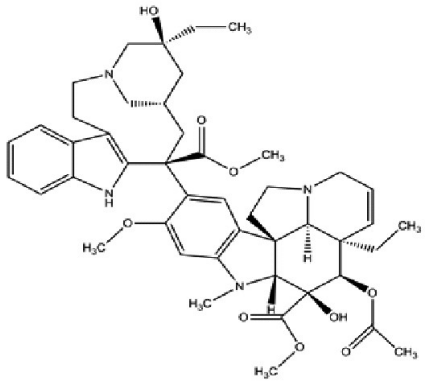
(III) Introduction

Catharanthus roseus is a therapeutic plant which has a place with the family Apocynaceae local and endemicto Madagascar. The plant is likewise referred to by the names, for example, *Vinca rosea, Ammocallis rosea and Lochnera rosea*. The plant has been put to customary use for the treatment of wide assortments of diseases worldwide since ages. The plant bears dynamic phyto constituents and displays different pharmacological exercises like enemy of diabetic, hostile to oxidant, against hypertensive, hostile to microbial, cytotoxic and so on. The plant creates a range of terpenoids indole alkaloids (TIAs) vinblastine and vincristine, the anticancer lead particles.

Alkaloids: Catharanthus roseus is known to be a wellspring of around 150 dynamic alkaloids out of which vincristine, vinblastine and vindiscline are of prime significance due to their utilization in the treatment of Cancer. Vinca alkaloids vincristine and vinblastine are utilized in chemotherapy with vincristine being utilized for intense lymphocytic leukemia, both Hodgkin and non-Hodgkin lymphomas and vinblastine being utilized as the significant segment in chemotherapy for germ cell, bosom, bladder and a few sorts of cerebrum malignancies. Vincristine and Vinblastine appeared in Figure 1 are hostile to tubulin drugs that demonstration by stifling the axle microtubule dynamicity in the phones during mitosis consequently capturing cell division and causing cell passing.

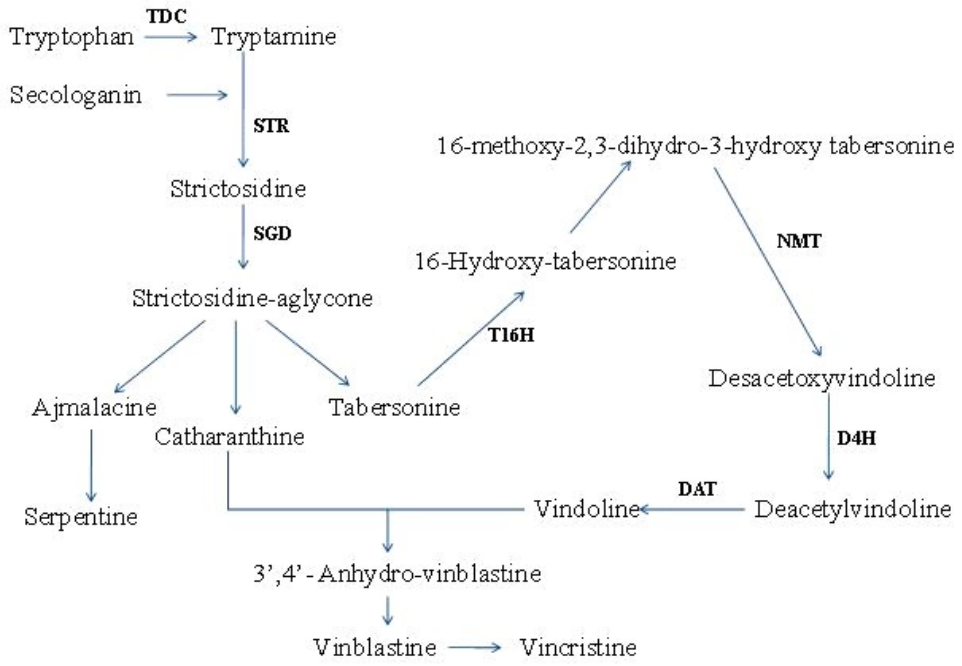


Vincristine



Vinblastine

Vincristine and vinblastine are the dimers formed by the coupling of monoindole alkaloids such as catharanthine and vindoline found abundantly in the aerial parts of the plant 9. The biosynthetic pathway of these alkaloids as in (Figure 2) has been found to be under strict developmental regulation in the plant 10.



Terpenoid Indole Alkaloid Biosynthesis in Plant System

The enzymes are abbreviated as follows: **TDC** – Tryptophan decarboxylase, **STR** – Strictosidine synthase, **SGD** – Strictosidine glucosidase, **T16H**– Tabersonine 16-hydroxylase, **NMT** - 16-methoxy-2, 3-dihydro-3-hydroxy tabersonine*N*-methyltransferase, **D4H** - Vindoline-4-hydroxylase, **DAT**- Deacetylvindoline*O*-acetyltransferase.

(IV) Aims & Objectives

- **Aim: -** Production of the secondary metabolites through plant tissue culture techniques.
- **Objective: -** Extraction of different alkaloids from *Catharanthus roseus.*

(V) Material & Methods

1. Source of explant- explants were collected from the field of ADITYA BIOTECH LAB RESEARCH PVT. LTD.
2. Callus induction-
   (a) Media preparation-
      - The Murashige and Skoog's (MS) basal medium was utilized for the investigation.
      - Stock answers for media arrangement were set up by utilizing required amounts of macronutrient and micronutrient for comfort.

- The pH of the medium was changed in accordance with 5.8 and cemented with 7% agar before autoclaving at 121˚C and 15 psi weight for 20 minutes.
- Media was put away at media extra space for atleast 3 days prior to inoculation.
- Nine different types of media were prepared with different concentration of hormones for the induction of callus.

Table 1: Showing Different Concentration of Harmones Used for Media Preparation

MEDIA TYPE	MS+2,4-D	BAP	NAA
Media 1	MS+.5mg/l	1mg/l	_
Media 2	MS+1 mg/l	_	_
Media 3	MS+1.5 mg/l	_	_
Media 4	_	1mg/l	0.5mg/l
Media 5	_	1mg/l	2mg/l
Media 6	MS+1 mg/l	1mg/l	_
Media 7	MS+2 mg/l	1mg/l	_
Media 8	MS+2 mg/l	_	_

Table 2: Showing Different Concentration of L-tryptophan

Media type	Concentration
Tryptophan media 1	150 mg/l
Tryptophan media 2	175 mg/l
Tryptophan media 3	200 mg/l

Media with different concentration of L-tryptophan

(b) Sterilization of explant-

Table 3: Different reagents used for the sterilization of plant.

0.1%$HgCl_2$ Mercuric Chloride	5% Sodium Hypochloride (SH)	Tween 80
5 minutes	15 minutes	5 minutes
10 minutes	20 minutes	_

- Dip the explant in water and wash thoroughly in running tap water.

- Dip the explant in Tween 80 (2 drops in 500ml water with vigorous shaking for 5 minutes.
- Transfer the explant in .1 % bavistin solution containing ampicillin (500mg/l) for 15 minutes.
- Inside laminar air flow wash once again with autoclaved distilled water.
- Dip the explant in 0.1% $HgCl_2$ for 5 minutes and 10 mins and some plants in 5% SH solution 15 & 20 minutes.
- Wash the explan 3 to 4 times with autoclaved distilled water.
- Inoculate the explant in the jar.

(c) Impact of amino acids on upgraded of cell development parameters and generation of absolute alkaloid, vinblastine and vincristine:

- L-tryptophan at the concentration of 150, 175 & 200 mg/l enhanced relative indole alkaloids production from leaf cell culture were investigated.
- The callus cultured in the MS medium were further subcultured in the medium containing L-tryptophan

(d) Extraction of alkaloids

- 100 gms of each plant material (callus) was ground and then extracted with methanol for 24 hours in a continuous extraction apparatus (soxhlet).
- Extract was filtered and then methanol was evaporated on a rotary evaporator under vacumm at a temperature of about 45˚C.

(e) Quantitative estimation (Thin layer chromatography)

- 50 ml aliquots applied on TLC plates.
- Dip in TLC solvent ethyl acetate: ethanol (4:1).
- Alkaloids were identified by Thin Layer Chromatography and color reaction with ammonium ceric sulphate.
- Mobility of alkaloids was compared with their RF and color reaction with ammonium ceric sulphate.
- With ammonium ceric sulphate vinblastine, vincristine will give lavender color with RF 0.35 for vinblastine, 0.25 for vincristine and 0.75 for catharanthine with blue color.

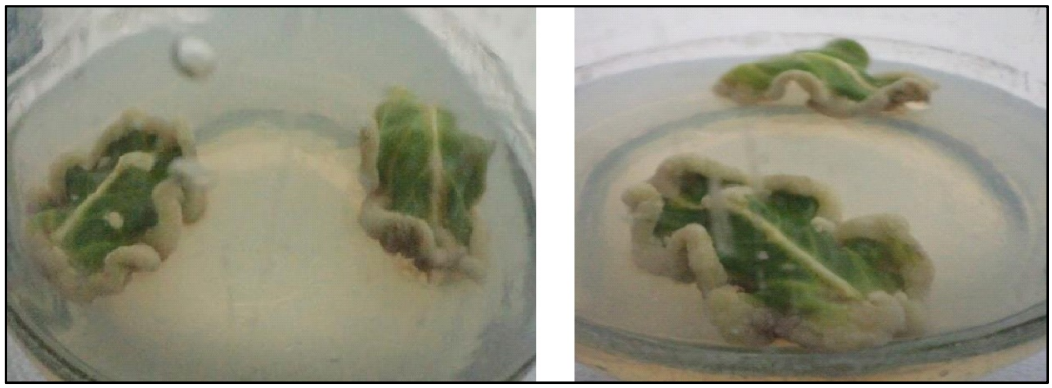


Figure A **Callus induction** **Figure B**

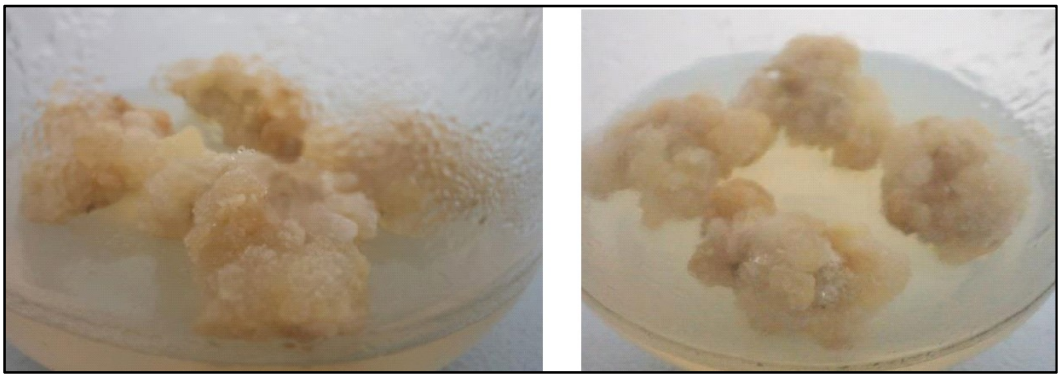


Figure C **Callus formed** **Figure D**

Figure E **Extraction of alkaloids through soxhlet** **Figure F**




Figure G Figure H Figure I Figure J

Set up for Thin Layer Chromatography (TLC)

(6) Result

Callus Induction

After four week of incubation, the callus were produced by leaf segments in the presence of auxin 2, 4-D, BAP and NAA.

MEDIA TYPE	MS+2,4-D	BAP	NAA	Response
Media 2	MS+ 1 mg/L	-	-	++
Media 3	MS+1.5mg/L	-	-	-
Media 4	-	1mg/L	0.5mg/L	++
Media 5	-	1mg/L	2mg/L	+
Media 7	MS+ 2mg/L	1mg/L	-	-
Media 8	MS+2mg/L	-	-	-

(+++) superior growth, (++) good growth, (+) poor growth (-) very poor

Very good amount of biomass of callus was found after four weeks of inoculation in the medium containing **0.5mg/l 2, 4-D and 1mg/l BAP** & in the medium containing **1mg/l 2, 4-D and 1mg/l BAP.**

Alkaloid assay from Callus Alkaloids

Alkaloids	RF	λ= 366	λ=254	CAS	Source (callus)	Source (leaf)
Serpentine	0.1	Blue	Blue	Non	Nil	Nil
Vincristine	0.25	Non	Dark	Lavender	Nil	Nil
Vinblastine	0.35	Non	Dark	Lavender	Nil	Nil
Catharanthine	0.75	Non	Dark	Blue	Nil	Nil

Table demonstrating the Removing factor (RF) and measures alkaloids shading response by TLC examination at UV (λ=366, λ =254).

The gauges Thin Layer Chromatography results demonstrated that Ajmalicine (RF= 0.95) at λ =366 nm, when respond with CAS cleanser gives apple-green and Vindoline (RF= 0.85) with at λ=366 when respond with CAS cleanser gives dim lavender shading.

No alkaloids were found in the callus culture due to less biomass of the callus sample for extraction because of all the callus got contaminated during third subculturing of the callus into tryptophan media.

By the extraction of dried leaf sample Vindoline (RF= 0.86) and Ajmalicine (RF = 0.94) were found to be present at different RF value when compared with the standard TLC result.

(7) Conclusion

From the present study done it can be concluded that the MS media supplemented with nutrient 0.5mg/l 2, 4-D and 1mg/l BAP is best for the callus induction. Three different reagents were used for the sterilization of explant and all were good, fewer amounts of contaminations were found in all the sterilization process after four weeks of inoculation.
When extraction was done with callus sample and with dried leaf sample no alkaloids were found in the callus sample but in dried leaf sample two alkaloids with some different RF is found i.e , Ajmalicine (RF = 0.94) and Vindoline (RF = 0.84).

Specific Achievement

Extraction of dried leaf showed different RF value for alkaloids Vindoline and Ajmalicine. RF for Vindoline should be 0.85 and for Ajmalicine it should be 0.95 but after the reaction with CAS it's showed RF 0.84 for Vindoline and RF 0.96 for Ajmalicine.

(VIII) References

1. Meenakshi Koul, Neha S. Lakra, Ramesh Chandra and Sheela Chandra catharanthusroseusand prospects of its endophytes: another road for creation of bioactivemetabolites IJPSR; Vol. 4(7): 2705-2716..2013.
2. Nergis Kaya and Cüneyt Akı Effect of plant development controllers on in vitro biomass changing in Catharanthus roseus (L) G Don Annals of Biological Research, 2013, 4(4):164-168 2013.
3. Dr. Negi RS Fast In-Vitro Callus Induction in Catharanthusroseus - A Medicinally Important Plant Used in Cancer Therapy. Research Journal of Pharmaceutical, Biological and Chemical Sciences ISSN: 0975-8585
4. Chinnamadasamy KalidassVeerabahu Ramasamy Mohan and Arjunan Daniel Effect of auxin and cytokinin on vincristine productionby callus culture of catharanthus roseus L.(apocynaceae), Tropical and Subtropical Agroecosystems, 12 (2010): 283–288
5. Ashutosh K. Verma, R. R. Singh and Seema Singh improved alkaloid content in callus culture of Catharanthusroseus. Botanica Serbica36 (2): (2012) 123 - 130
6. RizkitaRachmie Enhancement of Catharanthine Production in Catharanthus roseus Cell Cultures by Adding Tryptophan as Precursors International Conference on Mathematics and Natural Sciences (ICMNS) November 29-30, 2006,
7. Azra Ataei-Azimi, Babak Delnavaz Hashemloian Hassan Ebrahimzadeh and Ahmad Majd High in vitro generation of insect canceric indole alkaloids from periwinkle (Catharanthusroseus) tissue culture. African Journal of Biotechnology Vol. 7 (16), pp. 2834-2839, 18 August, 2008
8. Ian Scott, Hajime Mizukami, ToshifumiHikata and Siu-Leung Lee. Development of Cathranthine, Akuammicine and vindoline in CatharanthusroseusSusspension culture. Phytochemisty, 1980, vol.19, pp, 488-489.
9. Van Der Heijden R, Jacobs DI, Snoeijer W, Hallard D and Verpoorte
10. R. The Catharanthus alkaloids: Pharmacognosy and Biotechnology. Current Medicinal Chemistry, 2004, 11, 607-628 Arvind Verma, Into Laakso, Tuulikki Seppänen-Laakso, Aarre Huhtikangas and Marja-Liisa.Riekkola. A Simplified Procedure for Indole Alkaloid Extraction from CatharanthusroseusCombined with a Semi-engineered Production Process forVinblastine. Atoms 2007, 12, 1307-1315.

A Study of Virtual Classroom based on Information Technology based Infrastructure

Dr. Ashim Ranjan Sarkar[1]

(I) Abstract

In Virtual Classroom learners can learn individually. Outcome of this method gives high success ratio and flexible learning environment. It means Information-technology provides the features to manage courses in learner centric interactive environment. In our study, we investigated this ability of 30 registered in the class of Author. The methodology integrated an analysis of the groundwork of the study of students and evaluation of statistical data with their online past experience. We found that new structure of information's are required to cater online students to improve the skills.

(II) Key words

virtual classroom, online education

(III) Introduction

Conversion into non- conventional learning from traditional disciplines environments is the most tangible tasks of present education system [1]. It is feasible to educate and learn all subjects in classes conducted online non-concurrently. The main adorable feature is the use of information-technological resources to maintain the academic requirements.

(IV) Research methodology

We tried to fit the following hypotheses:

a) There may be a connection between academic progression and the students' course strategies [2];

b) Past online experience be able to induce a course opted by the student in the Virtual Class Room [3].

The methodology of investigation included: a) analysis of the groundwork of particular course [4], b) assessment of correlations in the Virtual Class Room [5].

[1] Asst. Prof., Dept. of CS & IT, Christ College, Jagdalpur

(V) Procedures and Outcomes

1. We investigate the students' behaviors in the Virtual Class Room during seven days before the examination. In this study, we classified the course of student strategies.

2. Then, we examined the correlations between a few indicators of students' course and their actual grades and the courses. This helped to know certain theory about the student in advance in the Virtual Class Room.

3. We examined the connection between the course strategies and their achieved grade-points. It was acceptable for coming across the connection between some approach for academic attainment. This might help to prophesy in online courses.

(VI) Methods of collecting statistical data

The BB software [7][8] used as an tool to gather data. It traced and archived all the activities of students' in the class during the exam. The BB record the arrival, departure time and activities in the VIRTUAL CLASS ROOM's content region. This tool generates various reports. These reports helped a lot of our research.

(VII) Data Representation

Online students' starts their attendance before than the course actually begins. The participants had a chance to login the site and without restraint discover there during seven days before the examination. This groundwork emerged to the most useful to discover their course strategies. When investigate the actions in class and examined data [8], we paying attention on the following indicators.

Time exhausted: Actual duration given for the Virtual Class Room[9] within appraise period indicate by ORNA. In the graph, the v. axis shows the names of students; the h. shows duration (in hours.)[10] Time given by the learner in the Virtual Class Room.

Stay in: The total number of stay-in/ visit into the VIRTUAL CLASS ROOM, which take place inside the examined duration, a constraint of action that we indicate by ORNA1. (Table 1)[11]

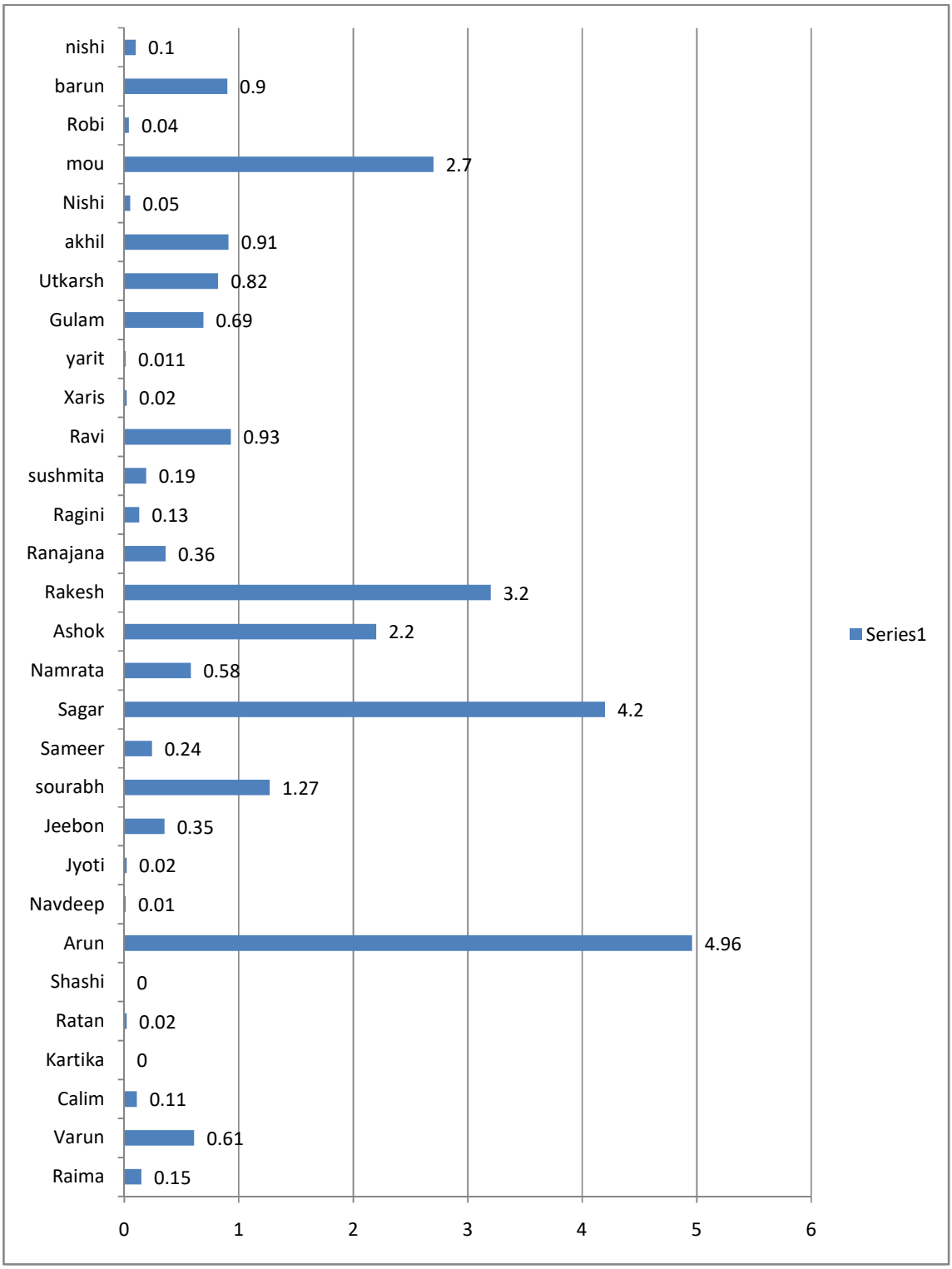


Figure 2. Graph of time spent by students

(VIII) Analysis of Data

Times given in the VIRTUAL CLASS ROOM before the examination had start presented students' ORNA[15].

Table1 : Visiting frequency of the VIRTUAL CLASS ROOM

	Content area					∑	Ratio
Name	BB help	C. Policy	Course Info	CW by W	Syllabus	a,b,c,e	d/f
	a	**b**	**c**	**d**	**e**	**f**	**g**
Raima	0	0	3	10	1	4	2.5
Varun	0	0	0	1	1	1	1.0
Calim	0	0	0	0	1	1	0.0
Kartika	0	0	0	0	1	1	0.0
Ratan	0	0	10	51	3	13	3.9
Shashi	0	0	1	15	0	1	15.0
Arun	0	0	0	13	2	2	6.5
Navdeep	0	3	0	0	0	3	0.0
Jyoti	0	0	9	31	3	12	2.6
Jeebon	0	0	2	44	2	4	11.0
sourabh	0	0	2	55	1	3	18.3
Sameer	0	0	3	3	0	3	1.0
Sagar	0	0	15	108	3	18	6.0
Namrata	0	0	0	4	1	1	4.0
Ashok	0	0	0	1	3	3	0.3
Rakesh	0	0	7	7	2	9	0.8
Ranajana	0	0	1	39	2	3	13.0
Ragini	0	0	6	21	3	9	2.3
sushmita	0	0	5	13	1	6	2.2
Ravi	0	0	5	30	4	9	3.3
Xaris	0	0	0	4	3	3	1.3
yarit	0	1	6	10	3	10	1.0
Gulam	0	0	27	95	1	28	3.4
Utkarsh	0	0	0	3	1	1	3.0
akhil	0	1	8	29	2	11	2.6
Nishi	0	0	0	3	2	2	1.5
mou	0	0	0	4	2	2	2.0
Robi	0	1	8	1	3	12	0.1
barun	0	0	0	1	3	3	0.3

nishi	0	0	0	3	3	3	1.0
			Average Ratio				**3.7**

After analyzing the data we have calculated the correlation (see table 2).

Table 2: Correlation

	ORN_A	ORN_{A1}	ORN_C	AA	EXP
Raima	0.36	14	2	86	1
Varun	0.03	1	1	82	1
Calim	0.00	1	1	80	1
Kartika	0.02	0	0	90	1
Ratan	0.58	64	3	87	0

We use, Pearson's correlation coefficients to find out the relationship between variables.

$$K = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - \sum x^2][n\sum y^2 - (\sum y)^2]}}$$

Where n is the students in a group; x=(x1…xn) and y=(y1…yn) are distributions of the variables[16][18][19].

Statistical Co-relational analysis displayed a constructive dependency among both directions of course activity: K (ORNA, ORNA1) = 0.83. Further, we shall use only ORNA. Indicators of groundwork correlates definitely with each other: K (ORNA, ORNC) = 0.5.

A positive statistical correlation found between course pursuit and academic accomplishment: K (ORNA, AA) = 0.4. No significant statistical correlation was found among course work and online familiarity: K (ORNA, EXP) = – 0.1. Sensible positive statistical correlation found between courses in the VIRTUAL CLASS ROOM and academic development: K (ORNC, AA) = 0.58. A negative statistical correlation was found between courses taken and online experience: K (ORNC, EXP) = – 0.48.

(IX) Results and Discussion

Course: Students explored the Virtual Class Room with diverse level of interest. Nobody walk around technical support provided by BB Help. Only out of 30 students 6 adapted with strategies for the online education. Even syllabus was not checked by few students.

In the meantime, several students were interested in the assignments by Week – it was stayed more frequently than all other affected areas of the VIRTUAL CLASS ROOM. It appears that they required for starting the first assignment.

Though, their annoyance to study the subject was not give back for lack of interest to mastering the aids of learning. In reality, they changed the problem.

Course and academic attainment: The hypothesis is the connections of among the course and academic up-liftment: K (ORNA, AA) = 0.4. A major course activity happened with a higher grade of final examination. The total time given in the VIRTUAL CLASS ROOM before the examination to ensure successful performance through the examination. Depended between content in the course and final grades is even more significant: K (ORNC, AA) = 0.6. These results ensures the more time is given for being aware the problem: its data, report, and the unknowns, – the advanced the probability that the dilemma will solve.

Course and online experience: There is no statistical correlation was found among course activity and experience: K(ORNA, EXPE) = – 0.1; and negative statistical correlation was discovered between orientation in the course material and experience K (ORNC, EXPE) = – 0.5. Thus, an general review of the VIRTUAL CLASS ROOM was commenced more often by novices than experienced students. There are several explanations of the result. By online experience we mean the number of taken courses. Though, the courses differ in their quality. Perhaps, some students attended classes in which they were not trained to explore the VIRTUAL CLASS ROOM. Also, need for course may depend more on the personality of student than experience. Self-controlled and disciplined students need less time than others.

(X) Reliability of the Result

Course endeavor overview and All User Activity inside Content Areas reports of 51 learners from the control inhabitants were analyzed. Their ORNA and ORNc exposed in a week before the exam were exposed.

Correlation between the students' ORNA and their AA for controls groups were considered. A dependency among the students' coursework and ranking in control groups was recorded. These outcomes are displayed in Table-3. The proximity of results of the trial group validate the reliability.

Table 3. The Correlation between ORNA and AA planned by Group Type.

The Correlation K(ORN_A,AA)		
The experimental group	The Control group1	The control group 2
0.41	0.42	0.34

In the groups, four coursework strategies were found: sensible, insular, poised, and careless. Their counts in the control groups were different; quality was same. Like in the experimental group, the students with sensible strategy form a majority in the organized-population.

Some students from these groups were not learn themselves in the VIRTUAL CLASS ROOM prior the examination. Like in the sample group, reverse orientation policy were found among students. Some students, who had the casual strategy, failed; remaining succeeded.

(XI) Conclusions

This study provides the teachers to better understand the requirements of student, issues, and course related approaches and to forecast the results in classes conducted online. Presence in the course makes online learning easier, and the absence tells a risk of failure. Students who used VIRTUAL CLASS ROOM before the examination they will definitely a step ahead from others.

Further to enhance the online courses' design will play an important role for the latent obscurities of many students' turning into the VIRTUAL CLASS ROOM. New structure of instructional base is required to help learners in the faculty of social sciences, notably, beginners, develop skills for getting proper knowledge of the VIRTUAL CLASS ROOM. A better consideration of the tools of learning allows for a more industrious study of their programmed subject.

(XII) References

[1] Bukaliya, R., Mubika, A.K. (2011). Teacher competence in ICT: Implications for computer education in Zimbabwean secondary schools. International Journal of Social Sciences and Education. Vol. 1, issue 4, 414-425
Dictionary. Retrieved on August 3, 2015 from http://dictionary.reference.com

[2] Hung, M.-L., Chou, C., Chen, C.-H., Own, Z.-Y. (2010). Learner readiness for online learning: Scale development and student perceptions. Computers & Education, 55, 1080-1090.

[3] How to Compute Pearson's Correlation Coefficient. Retrieved on August 3, 2015 from http://www.statisticshowto.com/articles/how-to-compute-pearsons-correlationcoefficients/

[4] Kaur, K., Abas, Z.W. (2004). An assessment of e-Learning readiness at Open University Malaysia. Retrieved on August 2004 from

[5] http://www.researchgate.net/profile/Zoraini_Wati_Abas/publication/228651396_An_assessment_of_elearning_readiness_at_Open_University_Malaysia/links/53cf3f180cf2fd75bc59b527.pdf

[6] Margaryan, A., Littlejohn, A. & Vojt, G. (2011). Are digital natives a myth or reality? Students' use of technologies for learning. Computer & Education, 56, 429-440.

[7] McVay, M. (2000). Developing a web-based distance student orientation to enhancestudent success in an online bachelor's degree completion program.

Unpublished practicum report presented to the Ed.D. Program. Florida: Nova Southeastern University.

[8] McVay, M. (2001). How to be a successful distance learning student: Learning on the Internet. New York: Prentice Hall.

[9] Polya, G. (1973). How to solve it. A new Aspect of Mathematical Method. Princeton, New Jersey: Princeton University Press.

[10] Prensky, M. (2001). Digital Natives, Digital Immigrants. On the Horizon. MCB University Press. Retrieved from http://www.marcprensky.com/writing/Prensky%20-%20Digital%20Natives,%20Digital%20Immigrants%20-%20Part1.pdf

[11] Rizzo, A., Boverly, T., Buckwalter, J.G., Schultheis, M., Matheis, R., Shahabi, C., Neumann, U., Kim, L. & Sharifzadeh, M. (2002). Virtual environments for the assessment of attention and memory processes: the virtual classroom and office. Proceedings of the 4th International Conference on Disability, Virtual Reality & Assoc. Technology. Veszprem, Hungary.

[12] Rizzo, A., Buckwalter, J.G., Shahabi, C. & Mitura, Roman (2004). Diagnosing attention disorders in a virtual classroom. Computer, Vol. 37 (6). DOI: 10.1109/MC.2004.23.

[13] Smith, P. (2005). Learning preferences and readiness for online learning. Educational Psychology, Vol. 25 (1), 3-12 So, T., Swatman, P. (2006). E-Learning readiness of Hong Long Teachers (2006).

[14] Proceedings of CollECTeR Europe 2006 conference. Basel, Switzerland, June 9-10. Ed. by Schubert P. and Risch D. Printed by University of Applied Sciences Northwestern Switzerland FHNW.

[15] The Free Dictionary. Retrieved on August 3, 2015 from http://www.thefreedictionary.com/orientation

[16] The Virtual Classroom Defined. Retrieved on August 3, 2015 from http://thelearningexplosion.blogspot.com/2010/12/virtual-classroom.html

[17] Warner, D., Christie, G. & Choy, S. (1998). Readiness of VET clients for flexible delivery including on-line learning. Brisbane: Australian National Training Authority.

]18] Watkins, R., Leigh, D. & Triner, D. (2004). Assessing readiness for e-Learning. Performance Improvement Quarterly, 17(4), 66-79.

[19] Yurdugul, H., Alsancak Sarikaya, D. (2013). The scale of online learning readiness: a study of validity and reliability. Education and Science. Vol. 38 (169), 391- .Retrieved on August 3, from http://search.proquest.com/openview/ c6b0a56d93852 05d7bf3759a9be dd68d/1?pq origsite =gscholar

Impact of Motivation on Employee Performance in Shivnath Hyundai Jagdalpur

Divya Dandwani[1]

(I) Abstract

Motivation is a vital concept that has been receiving considerable attention from academicians, researchers and practicing Human Resource managers. Inspiration is a fundamental component which urges individuals to give their best execution and help in accomplishing authoritative objectives. Employee motivation is the level of energy and commitment that company's employees possess for attaining goals and objectives of the organization. Motivation represents the willingness within an employee that helps improve organization's productivity and give the organization a competitive edge over its competitors.

The paper discuss on types and techniques of motivation. The method adopted is random sampling. This research paper aims to find out the influence of motivation on employees' poductivity. Thus the main focus of the research is to bring into light how motivation is a vital component that inspires employees to be more productive and helps to achieve organizational goals.

(II) Keywords

Motivation, Types of motivation, Factors affecting motivation.

(III) Introduction

In every firm managers tries to coordinate various resources so that their contribution is maximum in achieving organizational objectives. The exhibition of non-human elements like machines, and so forth relies upon the degree of innovation and the capability of the workers who use them. One needs to increase the efficiency of human beings for improving the performance of enterprises.

The word Motivation derives from the Latin word "Movere". The Latin word "Movere" means "To move", "To drive" or "To drive forward" etc. Motivation can be defined as a desire to perform an action that requires satisfaction. Inspiration is separated into two sections, directional, for example, coordinated towards a positive boost or away from a negative one, just as the enacted "looking for stage" and consummator "enjoying stage".

[1] Asst. Prof., Department of Management Studies, Christ College Jagdalpur, Bastar Distt. (C.G.), India

Incentive theories: Intrinsic and Extrinsic motivation

There are two types of motivation, *intrinsic* (internal or inherent) motivation and *extrinsic* (external) motivation.

Intrinsic motivation

Intrinsic motivation is defined as the self-desire to seek out new things and to participate in a competitive situation, to analyze one's capacity, to observe and to gain knowledge. Internal motivation is driven by an interest or enjoyment in the task itself, and exists within the individual rather than for some separable consequence.

Some examples of intrinsic motivation are as follows:

- **Acceptance:** Acceptance is one of the vital thing that one can practice in order to empower ourselves.
- **Curiosity:** This is a state where we are willing to discover more about something.
- **Honor:** We all need to respect and follow the rules, be trustworthy, reliable and to be ethical.
- **Independence:** Being independent can increase our self value and self-esteem which gives a sense of accomplishment.
- **Order:** One needs to be organized to attain organizational goals.
- **Power:** Power helps us have influence on other people.
- **Social contact:** Social interactions play a important role in every organisation.
- **Social Status:** Every individual has an urge to have high rank.

Extrinsic motivation

Extrinsic motivation is the behviour of individuals that is driven by external factors. Money is one of the examples of external motivation. Some other examples of extrinsic motivation are as follows:

- Employee of the month award
- Benefit package
- Bonuses
- Organized activities

Techniques to Increase Motivation

A. Financial Motivators

Financial motivators means monetary rewards. Incentives are nothing but rewards provided to employees in order to motivate them. Financial motivators are in the form of more wages and salaries, bonuses, profit-sharing, leave with pay medical reimbursements, company paid insurance of any of the other things that may be given to employees for improving efficiency of employees. Some economists and managers view money and financial incentives as essential motivators.

Some of the financial incentives are mentioned below:

1. Pay and Allowances
2. Incentive Pay
3. Gain Sharing
4. Profit Sharing
5. Stock Options
6. Retirement Benefits

B. Non-Financial Motivators

These motivators doesn't involve giving money directly to the employee but are in the form of better status, recognition, participation, job security etc.

Some of non-financial motivators are discussed below:

1. Recognition
2. Participation
3. Status
4. Competition
5. Job Enrichment
6. Terms of Employment
7. Communication Flow
8. Working Environment

(IV) Objectives of the Study

1. To study the impact of motivation on employees performance of Shivnath Hyundai.
2. To determine the factors that increase employees motivation in Shivnath Hyundai.
3. To study various techniques that motivates employees of Shivnath Hyundai.
4. To suggest strategies to improve employees performance of Shivnath Hyundai through motivational techniques.

(V) Research Methodology

Sources of data collection

(1) Primary source of data collection: - In this research the main sources of primary data includes filling questionnaires, interview, observation, etc.

(2) Secondary source of data collection: - In this research the various sources of secondary data for the study include library, websites such as **www.ShivnathHyundai.com**, books, journals, newspapers, magazines as **Hyundai EDGE Magazine, New HORIZONS 67** etc.

Research Methodology

Sampling Method	-	Random Sampling
Sampling unit	-	Employees of Shivnath Hyundai
Source of data	-	Primary and Secondary data
Sample size	-	60 employees from all departments of Shivnath Hyundai
Area of Study	-	Jagdalpur
Research Design	-	Exploratory cum Descriptive

Variables

Independent	-	Sex, Age, Income
Dependent	-	Employee motivation

CATEGORY OF EMPLOYEES:

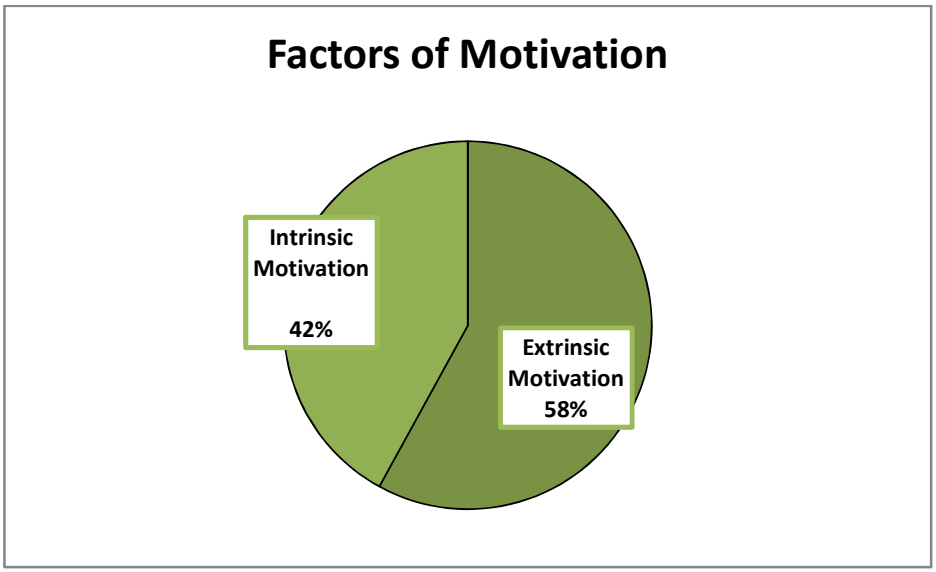
- Executive
- Managerial
- Lower level staff

For executives and managerial level data are collected through interview and observation method. For lower level staff questionnaire method for data collection is used.

Title	Information
Number of employees	60
Website	www.ShivnathHyundai.com
Magazines	Hyundai EDGE Magazine, New HORIZONS 67 etc.
Name of some cars	Hyundai Eon, Hyundai Grand i10, Hyundai elite i20 etc.

(VI) Analysis and Findings of the Study

1. Various Factors of Motivation: Intrinsic and Extrinsic

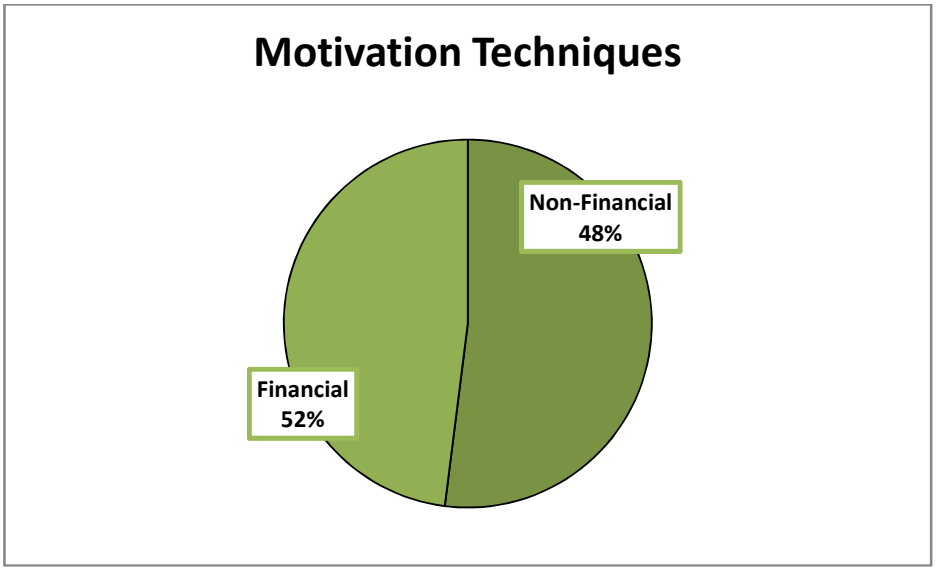


Inference: The study found that 58% of the employees are motivated by extrinsic factors and rest 42% are motivated by intrinsic motivation.

2. Motivation leads to job satisfaction of employees.

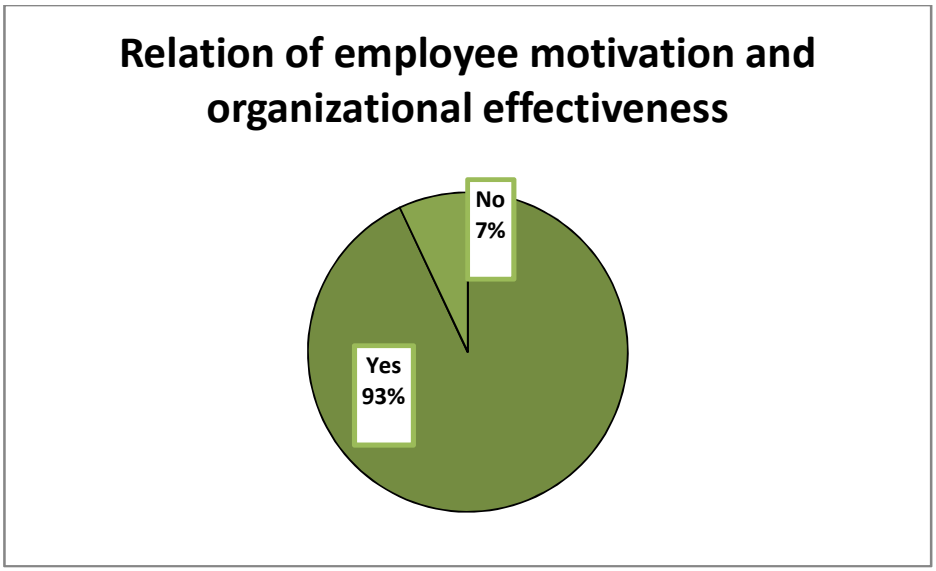
Inference: The study found that 86% of the employees say motivation gives job satisfaction to them and 14% disagrees.

3. Techniques that motivates employees.



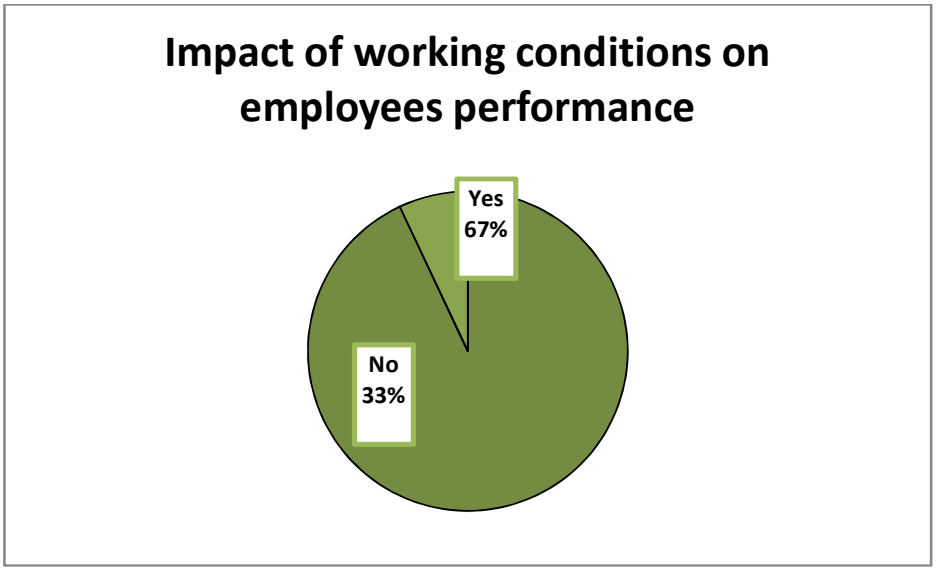
Inference: The study found that 52% of the employees say financial incentives motivate them and rest 48% says non-financial incentives motivates them.

4. Relation of employee motivation and organizational effectiveness.



Inference: The study found that 93% of the employees view that employee motivation has a direct relation to organizational effectiveness.

5. Impact of working conditions on employees performance.



Inference: The study found that 67% of the employees are of the view that working conditions motivates employees in an organization.

(VII) Conclusion

Motivation plays a vital role in all organizations. Motivated employees act as a valuable asset to the company. Motivation can be defined as a reason for employees' actions (or a reason) which leads an employee to act in a certain way.

A healthy working environment is the core element that will get a company to the top. It may be time consuming and challenging for managers to encourage employee motivation at the workplace. But, in order to increase the volume of production, management needs to encourage a positive working environment.

The paper concludes that managers must make certain that employees feel that their work and efforts plays an important role to the company's success. There are both internal and external sources that motivates the employees. Management must have an 'open-door' policy. There are both monetary and non-monetary factors which motivates employees for better performance. In order to be a great workplace leader, leader must be capable of motivating team members. All people are different, and so one might have to be aware of a few different ways to motivate the employees.

(VIII) Findings/ Suggestions of the Study

(1) Findings:

On the basis of my research, findings of my study are as follows:

- Employee Participation results in motivating the employees and provide knowledge valuable for the organizations success. Participation helps to give a sense of affiliation and accomplishment.
- Employees are influenced by their fellow workers.
- Employees are motivated both by intrinsic and extrinsic sources.
- Empowerment and recognition of employees in a firm encourages more motivated among the employees.
- There are various financial and non- financial incentives to motivate the employees.
- To increase employee productivity, management must try to build positive workplace environment.
- There exists a positive relationship between employee motivation and organizational effectiveness.
- These days organizations focus more on non-monetary employee motivation rather than tangible or monetary rewards.
- Motivation helps in reducing labour turnover and absenteeism rate.
- Motivation helps in reducing wastages, accidents, complaints and grievances.
- The research found that motivation results in increased productivity.
- Motivation satisfies both individual as well as group goals of employees in an organization.

(2) Suggestions:

The study has the following suggestions:

- Managers should try to understand that every individual is different from the other.
- Managers must try to value good work of employees in public and criticize the bad work privately.
- Superiors should have trust in employees.
- Employees should be encouraged to participate in decision making.
- Two-way communication to be followed.
- Managers must give challenging roles to employees.
- Managers should listen to the subordinates very attentively.
- Mangers should focus on giving suggestions rather than giving order.
- Survey and feedback method can be used by the management in order to solve the problems that the employees face.
- Mangers must provide a congenial work environment to employees.

(IX) References

1. Ahmad, M. Wasay, E. & Malik, S. (2012). Impact of Employee Motivation on Customer Satisfaction: Study of Airline Industry in Pakistan: Interdisciplinary Journal of Contemporary Research in Business, Institute of Interdisciplinary Business Research, Vol.4, No. 6.
2. Amjad Ali, Li Zhong Bin, Huang Jian Piang, Zulfiqar Ali (2016), The Impact of Motivation on the Employee Performance and Job Satisfaction in IT Park (Software House) Sector of Peshawar, Pakistan, International Journal of Academic Research in Business and Social Sciences, Vol. 6, No. 9ISSN: 2222-6990.
3. Azar, M & Shafighi, A. (2013). The Effect of Work Motivation on Employees' Job Performance: International Journal of Academic Research in Business and Social Sciences, Vol. 3, No. 9 ISSN: 2222-6990.
4. C.B.Mamoria, S.V. Gankar, Human Resources Management.
5. C.R. Kothari, Research Methodology Methods and Techniques
6. Chughtai (2008): Impact of Job Involvement on Role Job performance and organizational citizenship behaviour, September 1, 2016, Vol. 4, No. 3.
7. David A. De Cenzo, Human Resources Management.
8. Elizabeth Boye Kuranchie-Mensah, Kwesi Amponsah-Tawiah (2016), University for Development Studies, University of Ghana (Ghana), Employee Motivation and Work Performance: A Comparative Study of Mining Companies in Ghana, Journal of Industrial Engineering and Management, JIEM,– 9(2): 255-309 – Online ISSN: 2013-0953 – Print ISSN: 2013-8423.
9. Gary Dessler, Human Resources Management
10. Irum Shahzadi, Ayesha Javed, Syed Shahzaib Pirzada, Shagufta Nasreen, Farida Khanam (2014), Impact of Employee Motivation on Employee Performance, European Journal of Business and Management, ISSN 2222-1905 (paper) ISSN 2222-2839 (online) Vol.6 No.23.
11. James R. Lindner (May 2011), The Ohio State University, Understanding Employee Motivation, Journal of Extension, 3(5), pp. 76-79.
12. Judy Cameron (Sept. 1, 1994), Rewards and Intrinsic Motivation, Vol 64, Issue 3.
13. K Aswathappa, Human Resources Management.
14. L M Prasad, Principles and Practice of Management.
15. L.M.Prasad, Human Resources Management, Sultan Chand & Sons.
16. Maryam T. Abbah (Apr. 2014), Employee Motivation: The Key to Effective Organizational Management in Nigeria, IOSR Journal of Business and Management, e-ISSN: 2278-487X, p-ISSN: 2319-7668. Volume 16, Issue 4. Ver. I, PP 01-08.

17. Masud Ibrahim (November 2015) , University of Education Winneba, Veronica Adu Brobbey , University of Education Winneba, Impact Of Motivation On Employee Performance The Case Of Some Selected Micro Finance Companies In Ghana, International Journal of Economics, Commerce and Management, Vol. III, Issue 11, ISSN 2348 0386.
18. Md. Shariful Islam, Md. Faisal-E-Alam (2015), Motivational Factors Influencing on Job Satisfaction: A Case Study on Private Commercial Banking Sector in Rajshahi, Bangladesh, Journal of Resources Development and Management, Vol.13, ISSN 2422-8397.
19. N. K Singh, Human Resources Management, Himalaya Publishing House.
20. Nicholas J Bentell, Human Resources Management.
21. P. Jyothi, P., Venkatesh, D.N., Human Resource Management.
22. P.Subba Rao, Personnel and Human Resources Management, Text and Cases, Himalaya Publishing House.
23. Quratul-Ain Manzoor, Department of Management Sciences, Impact of Employees Motivation on Organizational Effectiveness, European Journal of Business and Management, ISSN 2222-1905 (Paper) ISSN 2222-2839 (Online) Vol 3, No. 3.
24. R.S. Gupta, B.D.Sharma, N.S.Bhalla, Principles and Practice of Management, Kalyani Publishers.
25. Randall S Schuler, Human Resources Management.
26. Rao Subba P. (2005). Essential of HRM & Industrial Relationships, Pg (480 to 482).
27. S. K. Srivastava et.al, (2011), Role of motivation in higher productivity, Management Insight, Vol. 7, No. 1.
28. Seyed Abdol Rasoul Hosseini (2014), Factors Affecting Employee Motivation, Management and Administrative Sciences Review, Vol. 3, Issue. 4 (Special Issue), Pages 713 -723.
29. Seyed Abdol Rasoul Hosseini (March 2017), Factors Affecting Employee Motivation, Management and Administrative Sciences Review, Volume 5 Issue III, ISSN: 2321-9653.
30. Shashi K Gupta, Rosy Joshi, Organisational Behaviour.
31. Surbhi, S. (2012). Effect of Motivation on employee performance and Organizational Productivity. Journal of Applied Management & Computer Science, 1.
32. T.N. Chhabra, Human Resources Management, Concepts and Issues, Dhanpat Rai & Co.
33. V.S.Palaniammal, Arivuselvee. V. J, (March 2017), A Studyon Motivational Factors Affecting Employee's Work Performance at Greaves Cotton Limited, Ranipet, Tamil Nadu, International Journal for Research in Applied Science & Engineering Technology (IJRASET), Volume 5 Issue III, ISSN: 2321-9653.

34. Vinay Chaitanya Ganta (November-2014), Department Of Commerce And Management Studies, Motivation In The Workplace To Improve The Employee Performance, International Journal of Engineering Technology, Management and Applied Sciences, Volume-2, Isuue-6, ISSN 2349-4476, PP.221-230.

Web Site:

www.ShivnathHyundai.com

The Impact of Social Media on Social Life Style: A Case Study of College Students in Jagdalpur

Jay Shankar Sahu[1]

(I) Abstract

The impact of social media in our education and society today are not less than miracle. Especially when we talk about students they are more addicted to social media and their other applications. This study aims to assess the impact of social media on student in bastar region. This study deployed a quantitative research methodology involving questionnaire research among the 100 college students in Bastar. The finding includes how students think, interact, communicate, find love, and spend time and many more.

(II) Introduction

Social media is the future of communication. It includes an array of internet-based tools and platforms that increase and enhance the experience of sharing information. The platform is all about community-based input, contact, content-sharing, and alliance. This interactive medium has a multitude of interesting things like forums, micro-blogging, social networking, social bookmarking, wikis and podcasts. Studies show that online social networks like Twitter, Face book or watsapp have a great impact on youths' lives.

(III) Objectives

1. Determine the impact of social networking websites on the education of students in bastar.
2. To analyze the positive impact of these websites on the education of students in bastar.
3. To analyze the negative impact of these websites on the education of students in bastar.
4. To determine how many students used these websites for their education and how many use for another purpose.

Negative impact of Social Media on Education

There is lot of impact of social media in which negative impact keep important role in student's life. When it comes to negative impact of social media it put distance between the personal relationships of the students. It creates lot of misconception between them. They spend their lot of time in such media unnecessary. They loss their body language, personality development etc. Due to more using of social media student are not able for accuracy performance and other activity which is done offline.

[1] Asst. Professor, Christ College Jagdalpur, Bastar Distt. (C.G.), India

Positive Impact of Social Media

Some of the positive effects of social media on education:

World has became very small by using internet and social media it has verity of positive impact. With the help of social media students are able to communicate with each other very easy they can rich their feeling and thoughts easy to overall world in a second. They can share, upload, and compress their document easily with the help of this medium.

(IV) Methodology

1. **Collection of Samples - Samples** were collected from different colleges of Bastar region. Especially from urban area where social media sites and internet are accessed for various purposes.
2. **Analysis of Sample –** Analysis of sample is done by some social media related questions like use of internet, which device they often use for accessing social media and other social network site.
3. **Analysis of data through Direct Interaction –** To analyze the data we directly interact with students who directly or indirectly daily or seldom uses social networks site and internet and takes the benefit of these.

To achieve the impact of social media we asked the following questions to students.

(a) Which of these is your preferred kind of social media application?

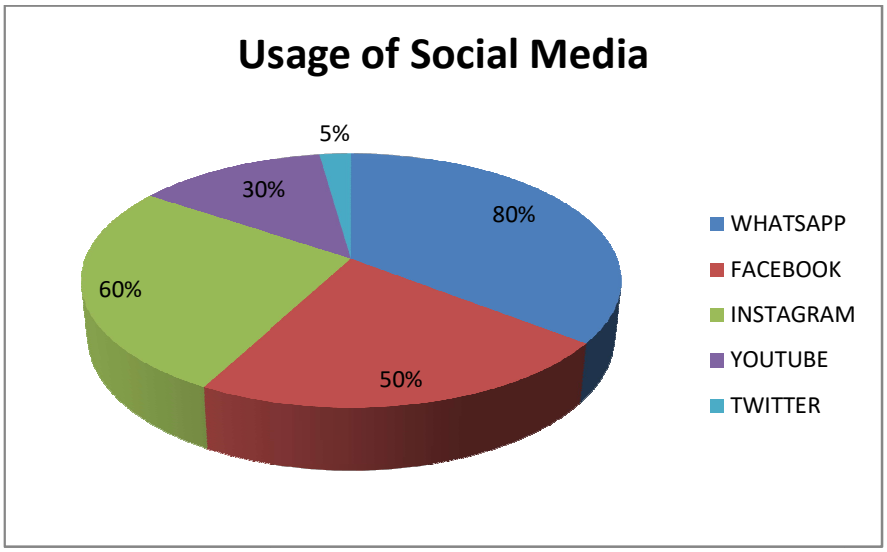
To find out the answer of this question we categorize it into five parts that uses different social media applications. Using this question we evaluate that how many users use which application to communicate.

(b) Social media is good for today's educations learning.

To find the answer of this question again we collect kind of answers through college students who are currently taking benefit directly or indirectly through social media.

(V) Analisis

1. Usage of Social Media



2. Responses on different parameters -

SN	Question	Strongly agree	Agree	Strongly Disagree	Disagree
1.	Social media is good for today's education learning.	20%	60%	10%	10%

SN	Question	Postitive	Negative
1.	The impact of social media is	70%	30%
2.	Social media sites creates unnecessary confusion	90%	10%
3.	Social media sites destroy students personal life	10%	90%
4.	Social media sites are time consuming	90%	10%
5.	Overall Social media is good for study	90%	10%

1.	How log student stay online	**<1 Hour**	**1 Hour**	**> 1 Hour**
		10%	40%	50%

(VI) Conclusion

Finally we reach in this conclusion that social media has become a guide for today's generation it has lot of impact which directly and indirectly put effect on students personal as well as educational life. Our research found that many students of the jagdalpur think that with the help of social media they connect with the overall world in a second and they can share their study materials to each other easily.

(VII) Appendix

Format of Questionaire

A Survey on
The Impact of Social Media on Social Life Style: A Case Study on College Students in Bastar

Name of Student…….. Class ……..

Age……. Contact Number ……..

Institution Name……

Note : Tick on suitable response(s) -

1. Which of the following is your preferred kind of social media application-
   ☐ Whatsapp ☐ FaceBook ☐ Instagram ☐ YouTube ☐ Twitter

2. Social media is good for today's Education learning -
   ☐ Strongly Agree ☐ Agree ☐ Strongly Disagree ☐ Disagree

3. The use of social media negatively affecting student's personal life -
   ☐ Yes ☐ No ☐ Some times

4. The use of social media creates unnecessary information and confusion on students mind -
   ☐ Yes ☐ No ☐ Some time

5. The use of social media impact is -
   ☐ Positive ☐ Negative ☐ Uncertain

6. Has the social media destroyed students personal life-
   ☐ Yes ☐ No ☐ I don't know

7. The use of social media is time consuming-
   ☐ Yes ☐ No ☐ I don't know

8. The use of social media is latest means for you to find love-
   ☐ Yes ☐ No ☐ I don't know

9. For how long you stay online .
   ☐ < 1 Hour ☐ 1 Hour ☐ > 1 Hour

10. Overall social media is good for learning-
    ☐ Yes ☐ No ☐ I don't know

References

[1] Joshua Ebere C. 2017 "The impact of social media on social life style: A case study of university female student", *Gender & Behaviour* (Vol. 15 No. 4) ISSN:1596-9231
[2] https://www.academiaapps.com/impact-social-media-education
[3] https://theknowledgereview.com/impact-social-networking-sites-youth

Awareness and Uses of Information Technology in a Rural Area of Bastar

Vandana Choure[1]

(I) Abstract

Information Technology has become core principal of success in today's world. We cannot imagine life without information technology. Without Information Technology a man cannot walk a step in today's life. Computer, Laptop, Mobile and other technical devices has become a core component of human body. Increasing use of this technology has made our life as fast as rocket. Information Technology is one of the important things for our social as well as personal life style. The objective of this research is to focus the awareness and uses of Information Technology in Bastar. This research includes 100 households and students of rural area of Bastar region. By doing this research we can get able to find out the awareness measures of technology in rural area of Bastar. Through this we can get knowledge about how many families and their members are aware of IT and their upcoming technology, how many are aware with mobile computing, cyber security etc.

(II) Keywords

Information Technology, Mobile Computing, Cyber Security.

(III) Introduction

Information technology is such a revolutionary initiative under which we can do our work system comfortably and in limited time. Information technology is the technology that helps in the management of information. This uses technology mainly in the collection and dissemination of information. In other words, information technology is synonym with modernization or development. Radio, Computer cellular phone, Satellite Communication, Optical fiber, Laser, Telephone etc, has jointly initiated information revolution around the world.

Information technology data recovery information is related to the collection of security changes exchanges study design and computer hardware and software application required for the execution of these task.

Information Technology means thing or techniques through which we become able to get information, Knowledge, News, and other important information. Information Technology has no limit. Today IT Domains in overall the world.

[1] Asst. Professor Christ College Jagdalpur, Bastar Distt. (C.G.), India

(IV) Advantages

(a) Economic

Information technology service is the basis of Finance system, information technology one appropriate technology for social and economic of backward countries.

In recent years, progress in information technology has caused many structural changes such as reorganizing of economics, globalization and trade extension, which leads to capital flow and enhancing information availability.

Information is empowerment with wealth.

(b) Politics

Political candidate use technology in many ways. Different the like of twitter, face book, YouTube are powerful communication media platform that can easily raise ratings of political candidates.

Information technology brings transparency in administration and government, it helps in reducing corruption.

Information technology is used in planning, policy making and decisions making.

Crowd sourcing is becoming a new weapon for policy and campaign among many political parties at present. They believe that this is the work that cannot be done by spending lakhes of rupees in advertising.

(c) Social Activity

Social media is basically linked to computers or any human communication or information exchange. The computer that is received through a tablet or mobile. There are many more websites and apps that make possible. Social media is becoming the largest medium communication and is gaining rapid popularity. Social media enables you to share ideas, content information and news with each other very fast.

Information and communication technology and sociology are well integrated and connected with each other. The society and the world are shrinking into village because computer and internet has made life easy to access anything present at any corner of the world. Before the use of information and communication technology sociology was limited into certain limited area.

(d) Education

Education technology aims to improve education. Technology should facility; learning process and increase performance of the educational system as it regard to effectiveness and efficiency.

Personalized and adaptive learning platform , software and digital devices are together creating countless new ways to modified education just like digital library , Unacademy

education portal, mobile based learning ,WI-FI connected open education resources, SWAYAM(study webs of active learning for young aspiring minds) , NPTEL (National Program on Technology Enhanced Learning) online course, Google Class, Google Scholar, etc.

(e) Science

Health IT is area of IT Involving the design, development, creation, use and maintains of information system for the health care industry. Just like Health Smart card (RSBY and MSBY), Aaushman Yojna, etc the schemes are being operated by the government whose benefits are the beneficial holders of the bank directly in there bank account.

IT has made the medical practice easy by making its incomparable contributions to the health sector. MRI, CT-Scan, X-RAY like health care tools etc. have been possible only under technology. Health criteria PROCTA (Hassel Free Appointment with Prime Doctor) 3 Step for better health-

- Get full today Checkup
- Connect with Doctor
- Order Medicine

(f) Bastar Net Project

Bastar Net project was announced on 15 august 2016 under IT in Naxal affected Bastar division of Chhattisgarh. 832 Kilometers of optical fiber cabling are being laid under this scheme. This ring method will be used in this network. Under which uninterrupted mobile and internet connectivity will be provided with alternative routes.

(V) Disadvantages

Where IT has played and important role in enhancing its knowledge, its loss cannot be denied. Hacking, spoofing and other such infamous activities on the internet have also become quite common.

If we use the internet in the online banking social networking site, then your personal information such as the name, address, mobile number, etc. can be misused.

Unlimited use of the internet enhances social distances.

Cyber crime, hacking, virus as the biggest hurdle in the field of IT and the IT industry. Everybody is playing a human game with blood clots of video game. How many children have committed suicide by playing games like Blue Whale, Momo. Playing a game called Blue Whale, 250 children around the world have died.

(VI) Objective of the Study

The main objective of the present study is as follows-

- To find out the awareness of Information Technology.
- To study the purpose and utilization of IT.
- To study the satisfaction of users about availability and coverage of IT.

(VII) Methodology

(a) Collections of Samples – Primary source were collected from questionnaire in which we include different villages of Bastar district especially from rural areas. Our study include 50 students and 50 household who uses mobile as well as who connected with internet and other information technology. Primary goal of our survey was to know the awareness of technology and other IT equipments.

(b) Analysis of Sample – After collecting of data through various places we analyze it by giving questions like- do you know Information Technology, awareness of Gmail account, use of Mobile Banking, use of Online Banking, use of Online Shopping, use of Mobile Phones, Desktop or Smart Phones, etc.

(c) Secondary Source - Secondary source were collected from direct interaction of the some students and households.

Questions through which we get the sample data-

1. Do you have any email account?
2. Do you use online Banking?
3. Do you use online shopping facility?
4. Do you know about digital locker?
5. Do you use paytm for payment and other purpose?
6. The use of technology is positive impact or negative.
7. Do you know about cyber security and cyber crime?
8. Information technology is good for today's education or not?
9. Which is your preferred social media application?
10. Overall Information technology is good or not, for learning?

(VIII) Finding and Discussion

Analysis of preferred social media application used by user.

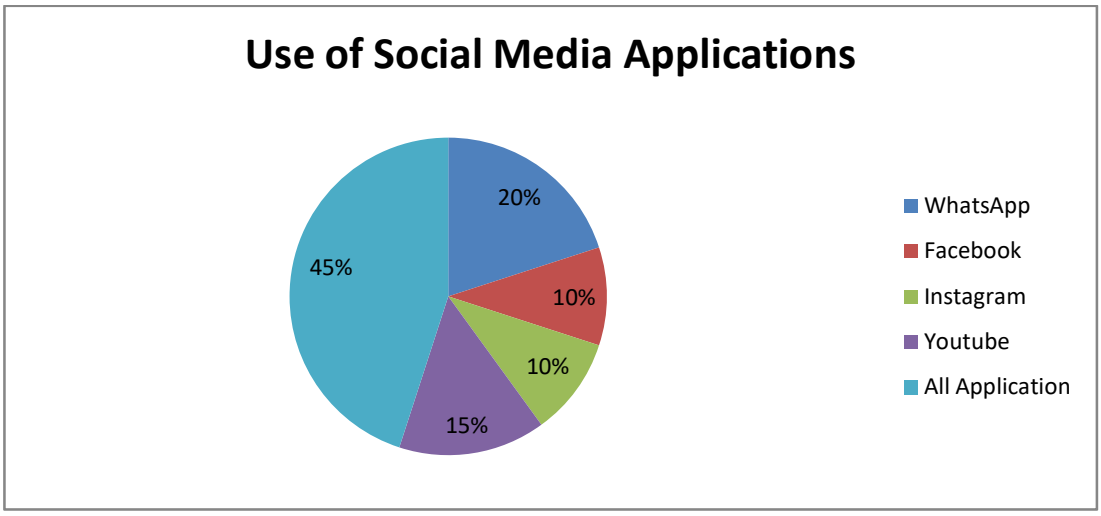


Table 1- Analysis of answers given by 100 users including Students and Female of rural area

QUESTION		Total	Yes	No
Do you have email account	Student	50	30	20
	Female	50	10	40
Do you use online Banking	Student	50	5	45
	Female	50	3	47
Do you use online shopping	Student	50	35	15
	Female	50	10	40
Do you know about digital locker	Student	50	6	44
	Female	50	-Nil-	50
Do you use paytm	Student	50	5	45
	Female	50	3	47
Use of technology			Positive	Negative
	Student	50	45	5
	Female	50	40	10
Do you know about cyber security	Student	50	10	40
	Female	50	3	47
IT is good for learning	Student	50	48	2
	Female	50	46	4
Overall IT is good for learning and education	Student	50	42	8
	Female	50	33	17

(IX) Result

After analyzing of sample of 100 students and households we found that 80% students have their own email ID reason behind this most of the students use smart phones and only 10% females have their email ID. Our next study was based on online banking in which we found that only 5% people use online banking or mobile banking facility. Next survey was for getting online shopping user in which we found 85% students uses online shopping and only 10% households uses this facility. Next factor was based on digital locker in which we got only 6% students know about it and none of the females are aware about it. This was the most poor part of the villagers. Next factor was Paytm, in which only 5% and 3% students and female uses this facility. The next survey was interesting, it was about use of technology have positive or negative impacts. In response, here 95% students are agree for positive response and 40% female also support for it. In cyber security only 10% students were aware about it and only 3% female were knowing about it. When we measure overall impacts of IT in learning and education, 92% students and 75 female supports for this.

(X) Conclusion

After analyzing all the factors, finally we reach in this conclusion that IT is one of the most important factor for today's generation. But when we talk about rural area of Bastar, most of the part are not aware about all the facility of technology. After analyzing the result, we found that, we should increase awareness programs as well as we should conduct IT training programs in rural area so that the people who are not aware about these facilities, can get touch with all these aspects. Government should also plan for improvement in rural area. We can organize awareness drive so that everyone may get the benefit from such programs.

(XI) References

[1] Peter Songan, "Awareness and Usage of Information Technology in a Rural Community of Bario"

[2] S. Thanuskodi, "Awareness and Use of ICT among Under Graduate Degree Students of Rural Areas in Tuticorin District, India: A Study"

[3] News paper –The Hindu/ cronical /Dainik bhaskar /Dandkarnya

[4] Magazine- Yojna/Vigyan pragati

[5] https://www.academiaapps.com/impact-social-media-education/

[6] https://theknowledgereview.com/impact-social-networking-sites-youth/

A Review Literature on

Genome Engineering Applications by CRISPR/Cas9

Dr. Anita Nair[1]

(I) Abstract

CRISPR-Cas9 technology is revolutionizing the field of genome editing. It can be used as a means to provide more efficient ways to create a meaningful change in the genome. Derived from components of a simple bacterial immune system, the CRISPR-Cas9 system permits targeted gene cleavage and gene editing in a variety of eukaryotic cells, and because the endonuclease cleavage specificity in CRISPR-Cas9 system is guided by RNA sequences, editing can be to anygenomic locus by engineering the guide RNA sequence and delivering it along with the Cas endonuclease to your target cell. The CRISP R-Cas9 system can be a powerful tool for genome editing in broad applications such as stem cell engineering, gene therapy, tissue and animal disease models, and engineering disease-resistant transgenic plants. The CRISPR (Clustered Regularly Interspaced Short Palindromic Repeat) restoration system is a promising option for genome editing. This review focuses on the system which can be used in the treatment of diseases, identifying the performance of defective genes in these diseases and use of CRISPR in understanding genetic and epigenetic diseases such as cancer. This system can also be used to create exact mutations in different cell lines to model the cancers. This type of modeling can lead to a better understanding of cancer and the ability to develop effective drugs.

(II) Keywords:

CRISPR, CRISPR-Cas9, genome editing

(III) Introduction

Gene editing technology is a new tool that can be used to introduce targeted modifications into the genome. There are three technologies for gene editing: Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Palindromic Repeats (CRISPRs) with CRISPR-associated (Cas) nucleases. Each of these systems is characterized by an adaptable sequence-specific DNA binding domain and a nuclease domain that creates a double-strand cleavage. The sitespecific DNA binding domains of the ZFN and TALEN systems are based on chimeric protein, whereas the CRISPR-Cas system utilizes an RNA molecule. [1]

[1] Asst. Prof., Dept. of Life Science, Christ College Jagdalpur, Bastar Distt. (C.G.), India

CRISPR together with Cas proteins form the CRISPR-Cas system[2], which is the newest gene editing method. CRISPRs constitute a family of short DNA repeats that are important components of the adaptive immune system in bacteria and archaea. These elements protect the microbes against various viral invasions. Cas proteins have functional domains that are similar to nucleases, helicases, polymerases, and polynucleotide-binding proteins[3]. Originally, the CRISPR-Cas system was divided into eight subtypes[4]. However, a new system is introduced because the previous classification did not take into consideration the distant relationships between various Cas proteins[5]. In this new classification, the CRISPR-Cas system is divided into three different types. The type I and III systems involve the specialized Cas endonucleases which process the pre-crRNAs and once mature, the crRNA will assemble into a large Cas protein complex. The complex is capable to recognize and cleave nucleic acids complementary to the crRNA[6]. The type II, which discuss here, CRISPR-Cas9 system is characterized as a small RNA-based immune system of bacteria and archaea[7], and recently it was developed for efficient genome engineering[8]. Cas9 is an RNA-guided DNA nuclease enzyme that provides an effective means of introducing targeted loss-of-function mutations at specific sites in the genome[9] by generating DNA Double Stranded Breaks (DSBs) at specific genomic loci. This system is easy to design, highly specific, efficient, and well-suited for high- throughput and multiplexed gene editing for a variety of cell types and organisms [10].

Type II CRISPR systems require only one protein, Cas9, to scan, bind and cleave the target DNA sequence[4]. Details of the Type II CRISPR/Cas9 system are shown in Figure 1. The genomic CRISPR locus is comprised of three components: the trans-activating CRISPR RNA (tracrRNA) gene, the Cas gene, and the CRISPR repeat and spacer sequences[5]. These are transcribed into tracrRNA, Cas9 protein, and pre-crRNA. Following transcription, the tracrRNA and pre-crRNA are stabilized by Cas9 and base pair, and RNase III processes the pre-crRNA into crRNA by cleaving it at the repeats (39). This dependence on RNase III likely explains why Type II systems are found in bacteria and not archaea, as RNase III is not found in archaea The crRNA:tracr RNA:Cas9 complex forms the active crRNA-guided endonuclease. Fig. 1.

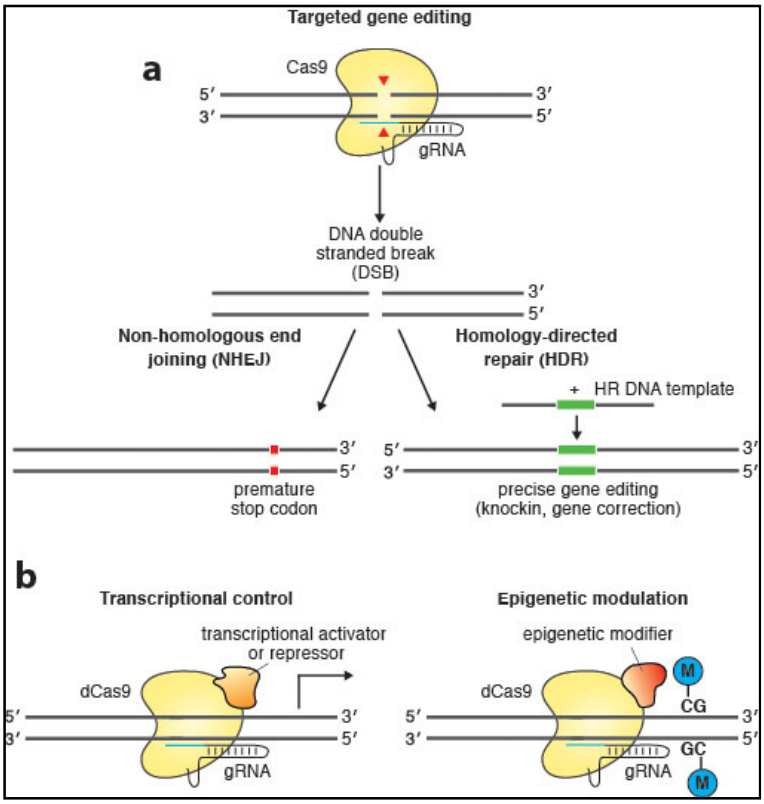


Figure 1: Genome editing applications of CRISPR-Cas.

(a) Targeted gene editing of the nuclease Cas9 guided by sgRNA.

(b) Cas9 with inactivated nuclease activity (dead Cas9, dCas9) fused to functional domains for transcriptional control and epigenetic modifications.

The CRISPR-Cas system is found in bacteria The CRISPR-Cas system is part of the immune system of the bacterium *Streptococcus pyogenes*. Similar systems have been discovered in 90% of the Archaea (ancient bacteria which have no cell nucleus like other bacteria, but in which the transcription of genes appears to proceed in the same way as in eukaryotes, which do have a nucleus) and 40% of bacteria.[10][11] [12] The CRISPR-Cas system consists of two RNA molecules and a protein. The first RNA molecule can bind to a specific DNA sequence of 20 nucleotides, the second RNA molecule binds to the first, and then the Cas protein can bind. When a Protospacer Adjacent motif (PAM) is present next to the sequence to which the first RNA molecule binds, the Cas9 protein cuts each strand of the double helix DNA molecule, thus causing a double strand break in the DNA. To make these cuts the Cas9 protein has two different nucleases, one for each strand of the DNA. The PAM sequence consists of the base sequence NGG, in which N can be any of the bases.

Genome Engineering using the CRISPR-Cas9 system

The CRISPR-Cas system makes it possible to introduce specific mutations or genes into a genome by means of homologous recombination (HDR). During the modification a DNA molecule must be introduced at the same time as the sgRNA and the Cas9. This DNA must have sequences on either side of the new sequence that are very similar to the sequences on either side of the double strand break.[13] In addition, CRISPR-Cas can be used to introduce small insertions and deletions (indels) in the genome, because a break in the DNA will be repaired by the NHEJ mechanism. Non-homologous end-joining (NHEJ) Another way of repairing a double strand break involves joining together the broken strands of DNA, which is called non-homologous end-joining. This requires little or no sequence homology and often results in deletions or insertions at the site of the break. [14],[15],[16]

Recent Advances in Genome Editing Using the CRISPR/Cas 9 *System*

In the recent past, researchers have explored CRISPR/Cas9 gene editing technology to combat HIV infection. Latently infected $CD4^+$ T cell lines, primary $CD4^+$ T cells, and induced human pluripotent stem cells (iPSCs) were incorporated with Cas9 and targeting gRNAs which prevents against the new infection by HIV-1[17] CCR5 and CXCR4 coreceptor genes have also been edited to render cells refractory to HIV-1 infection [19],[20]. However, the emergence of replication-competent viruses that are resistant to Cas9/sgRNA was also observed[21]**.** This technology was also employed to excise the integrated HIV-genome from the infected cells of preclinical animal models, including “humanized” mouse, using saCas9 and multiplex sgRNA[24], a step that is important towards fighting an HIV infection.

Yin et al. recently revealed that the CRISPR-Cas9 technology can be used to excise the HIV-1 DNA from the genome of a "humanized" mouse that carries HIV-1-infected human lymphocytes [25]. The authors also demonstrated that the gene-editing apparatus can be engineered to carry a set of multiple guide RNAs, all designed to efficiently excise integrated HIV-1 DNA from the host cell genome, a strategy that might well overcome a potential escape of mutated HIV-1 viruses. This proof-of-concept study indicates that the CRISPR-Cas9 technology may be useful in the design of new treatments directed against virally-induced malignancies, such as the Epstein-Barr virus (EBV)-induced Burkitt lymphoma[23] [26], human T-lymphotropic virus leukemia (HTLV)[22] [27], and Kaposi sarcoma-associated herpes virus (KSHV or HHV8) [28], as well as other life-threatening diseases induced by viruses such as the Ebola [29], and rabies[30]

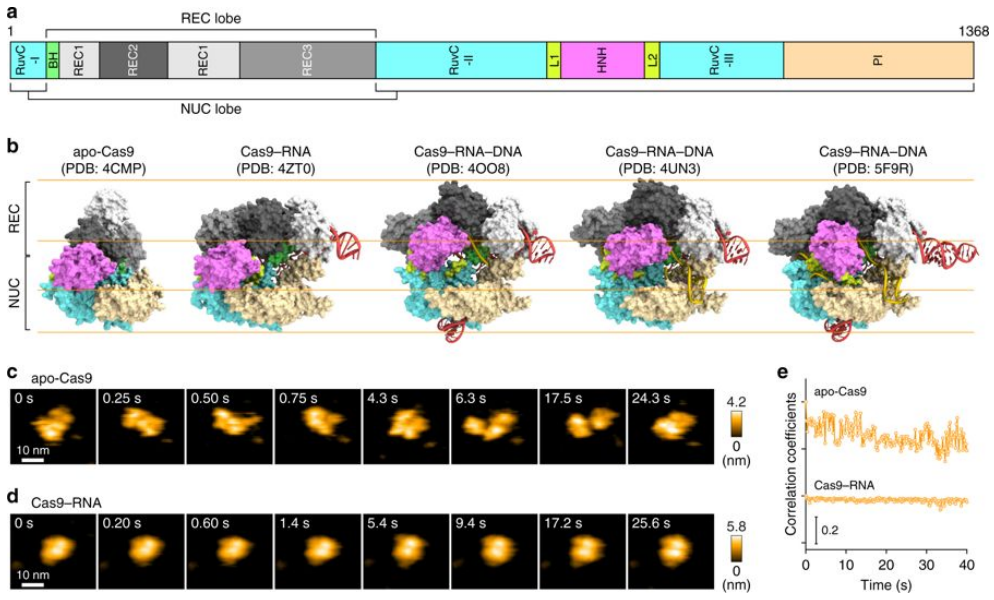
Genome engineering is particularly useful in drug discovery programs to identify genes that are responsible for a particular disease. The implementation of the CRISPR/Cas9 system,

however, has the potential to accelerate the identification and validation of high-value targets. Indeed, the rapid and efficient generation of precision disease models, cellular or whole animal, by CRISPR/Cas9 engineering, should have a positive impact on drug discovery as a faster route for functional drug screening through the identification of target molecules whose activation or inhibition causes or prevents disease [31]. Fig 2

Clustered regularly interspaced short palindromic repeats/Cas9 screens are particularly suitable to detect gene deletions associated with drug resistance. Accordingly, cells that acquire resistance to the drug of interest are exposed to a pool of CRISPR/Cas9 gRNAs that target various genes such that there is only one guide per cell and one gene knocked out. Genes that confer drug resistance are identified via analysis of the cells that become sensitive to the drug exposure. Those genes identified as resistant to the drug can then be targeted with other drugs to avoid the emergence of resistance [32]. For example, the disruption of the HPRT1 gene through CRISPR/Cas9 editing generates cells' resistant to 6-thioguannie (a conventional anti-cancer drug) [33]; similarly, the homozygous C528S mutation in the XPO1 gene mediated by CRISPR/Cas9 editing confers resistance to selinexor [34].

Fig. 2

From: Real-space and real-time dynamics of CRISPR-Cas9 visualized by high-speed atomic force microscopy



HS-AFM observations of apo-Cas9 and Cas9–RNA. **a** Domain structure of *S. pyogenes* Cas9. BH, Bridge helix. **b** Crystal structures of apo-Cas9 (PDB: 4CMP)12, Cas9–RNA (PDB: 4ZT0)13, Cas9–RNA bound to its single-stranded DNA target (PDB: 4OO8)15, Cas9–RNA bound to a partial DNA duplex (PDB: 4UN3)14 and Cas9–RNA bound to its dsDNA target (a Cas9 R-loop complex) (PDB: 5F9R)16. The guide RNA and the target DNA are colored red and yellow, respectively. The PAM is colored purple. The 98-nt guide RNA (PDB: 4OO8) was used for HS-AFM observations. **c**, **d** Sequential HS-AFM images of apo-Cas9 (**c**) and Cas9–RNA (**d**) on the AP-mica surface. The color (from black to white) corresponds to the height. The scale bars are 10 nm. **e** Time courses of correlation coefficients between the sequential HS-AFM images of apo-Cas9 and Cas9–RNA

(IV) Applications of CRISPR technique

- Regulatory pathways in bacterial and mammalian cells have been developed using transcriptional regulatory systems on the basis of CRISPR-Cas9. The progress made in transcriptional regulation by CRISPR allows multiple genes to be set simultaneously.
- CRISPR can remove the wrong exon so that the duplicator machine can produce a shorter version of dystrophin functioning like its normal version. sgRNA and Cas9 were transferred by an adenovirus carrier into rat muscle cells; then the wrong exon was removed using the CRISPR system. These results are promising towards finding proper treatments for Duchenne muscular dystrophy in the future correction of dystrophin mutations that cause Duchenne muscular dystrophy.
- Genome editing of the HIV co-receptors CCR5 and CXCR4 by using CRISPR-Cas9 has been proposed as a potentially safe and effective strategy to achieve treatment by protecting CD4(+) cells from HIV-1 infection using DNA editing, scientists injected engineered CRISPR to *Anopheles* mosquitoes, which are one of the major malaria-carriers in Asia. The inserted DNA encoded engineered antibodies that attack the malaria parasites. In the laboratory, this feature was extended to 99.5 percent of the offspring from matings between modified and unmodified mosquitoes[35]
- The CRISPR system could also be used for personal medical applications, in which embryonic stem cells are edited with the CRISPR technique, and then, re-injected into the patient. In this method, each person is treated according to their genetic characteristics, and their faulty genes will be modified directly.
- By creating a mutation in the the RuvC-NHN domains of CRISPR-Cas9, researchers plan to create a modified enzyme that does not have endonuclease activity and will be used merely to help target otherenzymes [36]The targeted mutations created in Cas9 make the protein lose its endonuclease property, but preserve its ability to identify its DNA target location and to bind it due to the presence of sgRNA sequences. The use of this system is

to attach other enzymes to the Cas9 sequence so that it can bind to the target site with Cas9 and perform their specific enzymatic activity instead of cleaving the sequence [37]

- This versatile genome editing tool has recently received attention for its potential in cellular models of ALS. The applicability of the CRISPR/Cas9 system for studying a "disease in a dish" that employs the patient's own derived induced pluripotent stem cells (iPSCs) was highlighted. Mutihac et al. [38] from the University of Oxford applied the CRISPR/Cas9 system to confirm models of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

(V) Conclusions

CRISPR medical applications are emerging. Clinical research has recently focused on attempts to use this technique in the treatment of cancer or other diseases. In international meetings and conferences, numerous promises are made regarding the possibilities for treatment and fixing of genetic defects in human embryos using the CRISPR technique. Today, although this technique has triggered ethical disputes, it is a fact that we already live in a CRISPR world. So what distinguishes CRISPR? The answer lies in its competitive techniques, ZFNs and TALEN, which are currently used by many companies for selective changes of particular DNA sequences of interest for medical purposes in clinical trials. Compared to them, CRISPR is acknowledged to have the advantages of being less labour intensive and more cost effective. However, the most important expectation of this method in the near future is that it will revolutionize the field of medicine by providing means for the prevention or the treatment of diseases, especially genetic diseases. Unlike other methods of creation of transgenic organisms, the performance of this technique does not require interference of foreign DNA. Like many great scientific discoveries of mankind, the CRISPR technique has many ethical questions that are not easy to answer. Despite all its promises, this technique still needs various improvements until it could be considered safe enough to be used on humans.

The CRISPR/Cas9 system makes genome engineering technology feasible for application in many fields including human diseases. Current research on this innovative genome editing tool and strategies to improve its drawbacks has laid the groundwork for future clinical work in neurodegenerative diseases. It has opened new avenues for studying the complexity of neurodegeneration in both in vivo and in vitro model systems. The ability of the system to establish large animal models that recapitulate specific human diseases improves our understanding of complex mechanisms involved in disease pathology.

(VI) References -

1. The CRISPR-Cas9 System: A New Dawn in Gene Editing Nurul Husna Shafie*, Mohamed Saleem, Emmanuel Jairaj Moses, Siti Razila Abdul Razak, and Narazah Mohd Yusoff Journal of Bioanalysis & Biomedicine Journal of Bioanalysis & Biomed ci ni e ISSN: 1948-593X Volume 6(6): 045-048 (2014) - 047 J
2. Haft DH, Selengut J, Mongodin EF, Nelson KE (2005) A guild of 45 CRISPRassociated (Cas) protein families and multiple CRISPR/Cas subtypes exist in prokaryotic genomes. PLoS Comput Biol 1: 60.
3. Van der Oost J, Jore MM, Westra ER, Lundgren M, Brouns SJ (2009) CRISPRbased adaptive and heritable immunity in prokaryotes. Trends Biochem Sci 34: 401-407
4. Makarova KS, Haft DH, Barrangou R, Brouns SJ, Charpentier E, et al. (2011)Evolution and classification of the CRISPR-Cas systems. Nat Rev Microbiol 9:467-477.
5. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, et al. (2012) Aprogrammable dual-RNA-guided DNA endonuclease in adaptive bacterialimmunity. Science 337: 816-821
6. Mali P, Yang L, Esvelt KM, Aach J, Guell M, et al. (2013) RNA-guided human genome engineering via Cas9. Science 339: 823-826.
7. Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, et al. (2013) Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 31: 227-229.
8. Cong L, Ran FA, Cox D, Lin S, Barretto R, et al. (2013) Multiplex genomeengineering using CRISPR/Cas systems. Science 339: 819-823.
9. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, et al. (2013) Genomeengineering using the CRISPR-Cas9 system. Nat Protoc 8: 2281-2308.
10. Maeder ML et al. (2013). CRISPR RNA-guided activation of endogenous human genes. Nat. Methods 10: 977-979
11. Sorek R et al. (2008). CRISPR - a widespread system that provides acquired resistance against phages in bacteria and archaea. Nat. Rev. Microbiol. 6: 181-186
12. van der Oost J et al. (2009). CRISPR-based adaptive and heritable immunity in prokaryotes. Trends Biochem. Sci. 34: 401
13. Mali P et al. (2013). RNA-guided human genome engineering via Cas9. Science 339: 823-826
14. . Wyman C & Kanaar R (2006). DNA double-strand break repair: all's well that ends well. Annu. Rev. Genet. 40: 363-383
15. Hwang WY et al. (2013). Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat. Biotechnol. 31: 227-229
16. Wu Y et al. (2013). Correction of a genetic disease in mouse via use of CRISPR-Cas9. Cell StemCell13: 659-662

17. R. Kaminski, Y. Chen, T. Fischer, and E. Tedaldi, "Elimination of HIV-1 Genomes from Human T-lymphoid Cells by CRISPR / Cas9 Gene Editing," Nat. Publ. Gr, 2016. View at Google Scholar
18. . Ebina, N. Misawa, Y. Kanemura, and Y. Koyanagi, "Harnessing the CRISPR/Cas9 system to disrupt latent HIV-1 provirus," Scientific Reports, vol. 3, 2013. View at Google Scholar · View at Scopus
19. H.-K. Liao, Y. Gu, A. Diaz et al., "Use of the CRISPR/Cas9 system as an intracellular defense against HIV-1 infection in human cells," Nature Communications, vol. 6, article 6413, 2015. View at Publisher ·View at Google Scholar · View at Scopus
20. H. Kang, P. Minder, M. A. Park, W. Mesquitta, B. E. Torbett, and I. I. Slukvin, "CCR5 Disruption in Induced Pluripotent Stem Cells Using CRISPR/Cas9 Provides Selective Resistance of Immune Cells to CCR5-tropic HIV-1 Virus," Molecular Therapy - Nucleic Acids, vol. 4, p. e268, 2015. View at Publisher ·View at Google Scholar
21. Z. Wang, Q. Pan, P. Gendron et al., "CRISPR/Cas9-Derived Mutations Both Inhibit HIV-1 Replication and Accelerate Viral Escape," Cell Reports, vol. 15, no. 3, pp. 481–489, 2016. View at Publisher · View at Google Scholar · View at Scopus
22. Yin C, Zhang T, Qu X, Zhang Y, Putatunda R, et al. (2017) In Vivo Excision of HIV-1 Provirus by saCas9 and Multiplex Single-Guide RNAs in Animal Models. Mol Her 25: 1168-1186
23. Klein G (2009) Burkitt lymphoma--a stalking horse for cancer research? Semin Cancer Biol 19: 347-350.
24. . Ciminale V, Rende F, Bertazzoni U, Romanelli MG (2014) HTLV-1 and HTLV-2: highly similar viruses with distinct oncogenic properties. Front Microbiol 5: 398.
25. Dittmer DP, Damania B (2016) Kaposi sarcoma-associated herpesvirus: immunobiology, oncogenesis, and therapy. J Clin Invest 126: 3165-3175.
26. Rougeron V, Feldmann H, Grard G, Becker S, Leroy EM (2015) Ebola and Marburg haemorrhagic fever. J Clin Virol 64: 111-119.
27. Velasco-Villa A, Mauldin MR, Shi M, Escobar LE, Gallardo-Romero NF, et al. (2017) history of rabies in the Western Hemisphere. Antiviral Res.
28. Scott, A. How CRISPR is transforming drug discovery. Nature 2018, 5555, S10–S11. [CrossRef] [PubMed]
29. Smurnyy, Y.; Cai, M.; Wu, H.; McWhinnie, E.; Tallarico, J.A.; Yang, Y.; Feng, Y. DNA sequencing and CRISPR-Cas9 gene editing for target validation in mammalian cells. Nat. Chem. Biol. 2014, 10, 623–625. [CrossRef] [PubMed]
30. Neggers, J.E.; Vercruysse, T.; Jacquemyn, M.; Vanstreels, E.; Baloglu, E.; Shacham, S.; Crochiere, M.; Landesman, Y.; Daelemans, D. Identifying drug-target selectivity of small-

molecule CRM1/XPO1 inhibitors by CRISPR/Cas9 genome editing. Chem. Biol. 2015, 22, 107–116. [CrossRef] [PubMed]

31. Brophy JA,Voigt CA. Principles of genetic circuit design. NatMethods. 2014;11(5):508–520.[Crossref], [PubMed], [Web of Science ®], , [Google Scholar],
32. Jusiak B, Cleto S, Perez-Piñera P et al. Engineering synthetic gene circuits in living cells with CRISPR technology. TrendsBiotechnol. 2016;34(7):535–547.[Crossref], [PubMed], [Web of Science ®], , [Google Scholar].
33. Long C, McAnally JR, Shelton JM, et al. Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science. 2014;345(6201):1184–1188.[Crossref], [PubMed], [Web of Science ®] , [Google Scholar]
34. Liu Z, Chen S, Jin X, et al. Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4(+) T cells from HIV-1 infection. Cell Biosci. 2017 [cited 2017 Nov 14];7:47. DOI:10.1186/s13578-017-0174-2[Crossref], [PubMed], [Web of Science ®], , [Google Scholar]].
35. Ghorbal M, Gorman M, Macpherson CR, et al. Genome editing in the human malaria parasite *Plasmodium falciparum* using the CRISPR-Cas9 system. Nat Biotechnol. 2014;32(8):819–821.[Crossref], [PubMed], [Web of Science ®] , [Google Scholar]
36. Sánchez-Rivera FJ, Jacks T. Applications of the CRISPR-Cas9 system in cancer biology. Nat Rev Cancer. 2015;15(7):387–395.[Crossref], [PubMed], [Web of Science ®],, [Google Scholar]
37. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014;157(6):1262–1278.[Crossref], [PubMed], [Web of Science ®] , [Google Scholar]
38. Mutihac, R.; Ababneh, N.; Scaber, J.; Wade-Martins, R.; Cowley, S.; Talbot, K. Modelling amyotrophic lateral sclerosis (ALS) using mutant and CAS9/CRISPR-corrected motor neurons from patients with C9ORF72 mutations reveals disease-specific cellular phenotypes. J. Neurol. Sci. 2015, 357 (Suppl. 1), e48. [CrossRef]
39 Deltcheva, K. Chylinski, C.M. Sharma, K. Gonzales, Y. Chao, Z.A. Pirzada, M.R. Eckert, J. Vogel, E. Charpentier**CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III** Nature, 471 (2011), pp. 602-607

A Review Paper on

Protein-Protein Interaction: Method and Analysis

Rahul Singh Thakur[1]

(I) Abstract

Protein-protein interaction helps in predicting the protein function of various protein and . various genes and proteins realize resulting phenotype functions as a set of interactions. Various method like vitro and in vivo methods like affinity purification, Y2H (yeast 2 hybrid), TAP (tandem affinity purification), and so forth have their own limitations like cost, time, and so forth, and the resultant data sets are noisy and have more false positives to annotate the function of drug molecules. It include databases containing experimentally verified and computationally inferred physical and functional interactions. There are also tools for predicting interactions and for extracting information on interactions from the literature, and organism specific databases

(II) Keywords

Inslico, datasets; domain, phylogenetic, coimmunoprecipitation, protein arrays, high-throughput.

(III) Introduction

Protein-Protein Interactions (PPIs) has a enormous range of biological processes, including cell-to-cell interactions and metabolic and developmental control. This interaction is becoming one of the major objectives of system biology. Various contacts between the residue side chains are the basis for protein folding, protein assembly, and PPI. These contacts induce a variety of interactions and associations among the proteins. Based on their contrasting structural and functional characteristics, PPIs can be classified in several ways. On the basis of their interaction surface, they may be homo- or heterooligomeric; as judged by their stability, they may be obligate or nonobligate; as measured by their persistence, they may be transient or permanent. PPI may be a combination of these three specific pairs. Various interactions would form signaling pathways while permanent interactions will form a stable protein complex. The yeast 2-hybrid system (Fields and Song, 1989) was initially performed using a GAL4 DNA binding domain-fusion with the chosen bait protein to capture its prey (an interacting protein) from a library of proteins labelled with the activation domain of GAL4. When the two proteins interact, a beta-galactosidase reporter gene (with an upstream Gal4p binding site) is activated. Nowadays, a library of haploid yeasts expressing bait-activation domain fusions can be mated sequentially with clones from a library of opposite mating type yeasts expressing prey-binding domain fusions

(Uetz et al., 2000; Ito et al., 2001). In this set-up, the interaction causes expression of a gene (or genes) required for growth of the diploid on selective medium (e.g. HIS3, an auxotrophic marker). The development of other high-throughput strategies has allowed the use of this system to provide interaction networks for Escherichia coli bacteriophage Lambda (Bartel et al., 1999) and Helicobacter pylori (Rain et al., 2001). One important point to note though is that the datasets from the two global screens of yeast showed very few overlaps, indicating a high false negative rate for these approaches. This issue, and others, including standardisation of these experiments to allow comparison of interaction networks, are becoming important topics in proteomics. Another technique for detecting interaction partners of chosen proteins is phage-display, in which short oligonucleotides are inserted within a gene encoding a coat protein of a bacteriophage, so that each viral peptide displays a different peptide sequence (for a review see Rodi and Makowski, 1999). Libraries of these phage are then screened against the protein of interest, to identify those peptides which bind the protein. Certain display technologies are yeast display, in which the oligonucleotides are inserted into the genes for yeast surface proteins, and ribosome display (Hanes and Pluckthun, 1997), in which the translated protein emerges from the ribosome and can fold, but remains part of the ribosomal complex and associated with its mRNA, allowing its identification by Comparative and Functional Genomics. Phage display has been widely used for the selection and evolution of antibodies and this is the principal area to which ribosome display has so far been applied.

(IV) Materials and Method

These detection methods are categorically classified into three types, namely, in vitro, in vivo, and in silico methods. In invitro techniques, a given procedure is performed in a controlled environment outside a living organism. Certain method such as in vitro methods in PPI detection are tandem affinity purification, affinity chromatography, coimmunoprecipitation, protein arrays, protein fragment complementation, phage display, X-ray crystallography, and NMR spectroscopy.Such techniques as In vivo techniques, a given procedure is performed on the whole living organism itself. These in vivo methods in PPI detection are yeast two-hybrid (Y2H, Y3H) and synthetic lethality. Various methods such as Insilico techniques are performed on a computer (or) via computer simulation. It is sequence-based approaches, structure-based approaches, chromosome proximity, gene fusion, in silico 2 hybrid, mirror tree, phylogenetic tree, and gene expression-based approaches, summarized in the Table below –

Technique	Summary
Tandem affinity purification-mass spectroscopy (TAP-MS)	TAP-MS is based on the double tagging of the protein of interest on its chromosomal locus, followed by a two-step purification process and mass spectroscopic analysis
Affinity chromatography	Affinity chromatography is highly responsive, can even detect weakest interactions in proteins, and also tests all the sample proteins equally for interaction
Coimmunoprecipitation	Using a whole cell extract where proteins are present in their native form in a complex mixture of cellular components
Protein microarrays (H)	Allows the simultaneous analysis of thousands of parameters within a single experiment
Protein-fragment complementation	Protein-fragment complementation assays (PCAs) can be used to detect PPI between proteins of any molecular weight and expressed at their endogenous levels
Phage display (H)	These approach originated in the incorporation of the protein and genetic components into a single phage particle
X-ray crystallography	It enables visualization of protein structures at the atomic level and enhances the understanding of protein interaction and function
NMR spectroscopy	It can even detect weak protein-protein interactions
Yeast 2 hybrid (Y2H) (H)	Yeast two-hybrid is typically carried out by screening a protein of interest against a random library of potential protein partners
Synthetic lethality	Based on functional interactions rather than physical interaction
Ortholog-based sequence approach	These approach based on the homologous nature of the query protein in the annotated protein databases using pairwise local sequence algorithm
Domain-pairs-based sequence approach	Thees approach predicts protein interactions based on domain-domain interactions
Structure-based approaches	It predict protein-protein interaction if two proteins have a similar structure (primary, secondary, or tertiary)
Gene neighborhood	If the gene neighborhood is conserved across multiple genomes, then there is a potential possibility of the functional linkage among the proteins encoded by the related genes
Gene fusion	Gene fusion, which is often called as Rosetta stone method, is based on the concept that some of the single-domain containing proteins in one organism can fuse to form a multidomain protein in other organisms
In silico 2 hybrid (I2H)	The I2H method is based on the assumption that interacting proteins should undergo coevolution in order to keep the protein function reliable
Phylogenetic tree	It predicts the protein-protein interaction based on the evolution history of the protein

Phylogenetic profile	Predicts the interaction between two proteins if they share the same phylogenetic profile
Gene expression	It is based on the idea that proteins from the genes belonging to the common expression-profiling clusters are more likely to interact with each other than proteins from the genes belonging to different clusters

It is rapidly becoming established as a powerful means to detect proteins, monitor their expression levels, and probe protein interactions and functions. It is a piece of glass on which various molecules of protein have been affixed at separate locations in an ordered manner. It has seen tremendous progress and interest at the moment and have become one of the active areas emerging in biotechnology.

Predictions of PPIs have been carried out by integrating evidence of known interactions with information regarding sequential homology. This approach is based on the concept that an interaction found in one species can be used to infer the interaction in other species. However, recently, Hosur et al. developed a new algorithm to predict protein-protein interactions using threading-based approach which takes sequences as input, which predicts whether two proteins interact by combining a novel linear programming approach for interface alignment with a boosting classifier for interaction prediction. Guilherme Valente et al. introduced a new method called Universal In Silico Predictor of Protein-Protein Interactions (UNISPPI), based on primary sequence information for classifying protein pairs as interacting or noninteracting proteins. The hybrid methods which use a combination of properties like protein sequences, gene ontologies, and so forth. However, there are two different methods under sequence-based criterion-

(1) Ortholog-Based Approach. The approach for sequence-based prediction is to transfer annotation from a functionally defined protein sequence to the target sequence based on the similarity. Annotation by similarity is based on the homologous nature of the query protein in the annotated protein databases using pairwise local sequence algorithm. Several proteins from an organism under study may share significant similarities with proteins involved in complex formation in other organisms.

The prediction process starts with the comparison of a probe gene or protein with those annotated proteins in other species. If the probe gene or protein has high similarity to the sequence of a gene or protein with known function in another species, it is assumed that the probe gene or protein has either the same function or similar properties. Most subunits of protein complexes were annotated in that way. When the function is transferred from a characterized protein to an uncharacterized protein, ortholog and paralog concepts should be applied. There are the genes in different species that have evolved from a common

ancestral gene by speciation. In contrast, paralogs usually refer to the genes related by duplication within a genome. In broad sense, orthologs will retain the functionality during the course of evolution, whereas paralogs may acquire new functions. If two proteins—A and B—interact with each other, then the orthologs of A and B in a new species are also likely to interact with each other.

(2) Domain-Pairs-Based Approach- It is a distinct, compact and stable protein structural unit that folds independently of other such units. But most of times, domains are defined as distinct regions of protein sequence that are highly conserved in the process of evolution. As individual structural and functional units, protein domains play an important role in the development of protein structural class prediction, protein subcellular location prediction, membrane protein type prediction and enzyme class and subclass prediction.

Conventionally, protein domains are used for basic research and also for structure-based drug designing. In addition, domains are directly involved in the intermolecular interaction and hence must be fundamental to protein-protein interaction. Multiple studies have shown that domain-domain interactions (DDIs) from different experiments are more consistent than their corresponding PPIs. So, it is quite reliable to use the domains and their interactions for prediction of the protein-protein interactions and vice versa.

Gene fusion, which is often called as Rosetta stone method, is based on the concept that some of the single-domain containing proteins in one organism can fuse to form a multidomain protein in other organisms. This domain fusion phenomenon indicates the functional association for those separate proteins, which are likely to form a protein complex. It has been shown that fusion events are particularly common in those proteins participating in the metabolic pathway. This method can be used to predict protein-protein interaction by using information of domain arrangements in different genomes. However, it can be applied only to those proteins in which the domain arrangement exists.

Another important method for detection of interaction between the proteins is phylogenetic tree. The phylogenetic tree gives the evolution history of the protein. The mirror tree method predicts protein-protein interactions under the belief that the interacting proteins show similarity in molecular phylogenetic tree because of the coevolution through the interaction. The underlying principle behind the method is that the coevolution between the interacting proteins can be reflected from the degree of similarity from the distance matrices of corresponding phylogenetic trees of the interacting proteins. The set of organisms common to the two proteins are selected from the multiple sequence alignments (MSA) and the results are used to construct the corresponding distance matrix for each protein. The BLAST scores could also be used to fill the matrices. Then the linear correlation is calculated among these distance matrices. High correlation scores indicate the

similarity between the phylogenetic trees and therefore the proteins are considered to have the interaction relationship. The MirrorTree method is used to detect the coevolution relationship between proteins and the results are used to infer the possibility of their physical interaction.

A PPI network can be described as a heterogeneous network of proteins joined by interactions as edges. The computational analysis of PPI networks begins with the illustration of the PPI network arrangement. The simplest sketch takes the form of a mathematical graph consisting of nodes and edges. Protein is represented as a node in such a graph and the proteins that interact with it physically are represented as adjacent nodes connected by an edge. An examination of the network can yield a variety of results. For example, neighboring proteins in the graph probably may share more the same functionality. In addition to the functionality, densely connected subgraphs in the network are likely to form protein complexes as a unit in certain biological processes. Thus, the functionality of a protein can be inferred by spotting at the proteins with which it interacts and the protein complexes to which it resides. The topological prediction of new interactions is a novel and useful option based exclusively on the structural information provided by the PPI network (PPIN) topology. Some algorithms like random layout algorithm, circular layout algorithm, hierarchical layout algorithm, and so forth are used to visualize the network for further analysis. Precisely, the computational analysis of PPI networks is challenging, with these major barriers being commonly confronted :
(1) the protein interactions are not stable;
(2) one protein may have different roles to perform;
(3) two proteins with distinct functions periodically interact with each other.

(V) Result

The available methods are unable to predict interactions with 100% accuracy, computational methods will scale down the set of potential interactions to a subset of most likely interactions. These interactions will serve as a starting point for further lab experiments. The gene expression data and protein interaction data will improve the confidence of protein-protein interactions and the corresponding PPI network when used collectively.

(VI) Discussion

Recent developments have also led to the construction of networks having all the protein-protein interactions using computational methods for signal transduction pathways and protein complex identification in specific diseases.

(VII) References

1. P. Braun and A. C. Gingras, “History of protein-protein interactions: from egg-white to complex networks,” Proteomics, vol. 12, no. 10, pp. 1478–1498, 2012.
2. Y. Ofran and B. Rost, “Analysing six types of protein-protein interfaces,” Journal of Molecular Biology, vol. 325, no. 2, pp. 377–387, 2003.
3. I. M. A. Nooren and J. M. Thornton, “Diversity of protein-protein interactions,” The EMBO Journal, vol. 22, no. 14, pp. 3486–3492, 2003.
4. A. Zhang, Protein Interaction Networks-Computational Analysis, Cambridge University Press, New York, NY, USA, 2009.
5. M. Yanagida, “Functional proteomics; current achievements,” Journal of Chromatography B, vol. 771, no. 1-2, pp. 89–106, 2002.
6. Rain JC, Selig L, De Reuse H, et al. 2001. The protein-protein interaction map of Helicobacter pylori. Nature 409(6817): 211–215.
7. Rodi DJ, Makowski L. 1999. Phage-display technology – finding a needle in a vast molecular haystack. Curr Opin Biotechnol 10: 87–93.
8. Schwikowski B, Uetz P, Fields S. 2000. Anetwork of proteinprotein interactions in yeast. Nat Biotechnol 18(12): 1257–1261.
9. Uetz P, Giot L, Cagney G, et al. 2000. Acomprehensive analysis of protein-protein interactions in Saccharomyces cerevisiae. Nature 403(6770): 623–627.
10. Xenarios I, Rice DW, Salwinski L, Baron MK, Marcotte EM, Eisenberg D. 2000. DIP: the Database of Interacting Proteins. Nucleic Acids Res 28: 289–291.

A Review Paper on

Extraction and optimization of Protease Activity by Bacillus Subtitlis from Different Sources and Finding Out the Best Source for Optimized Production in Future

Neha Pathak[1]

(I) Abstract

Screening and isolation of protease producing strain of bacteria i.e. *Bacillus subtilis* were carried out by extraction from three different sources i.e. Soil, sea water, and degraded abattoir waste from of different locations. This paper gives a study of optimized production of protease from the strains of *bacillus subtilis* which shows different criteria of production due to varying sources. **Firstly,** the protease producing bacterial strain was isolated from sea water and identified by 16s rrna sequencing. Optimization revealed that the most suitable Nitrogen source was beef extract and carbon source was glucose. 7% nacl conc. Showed highest yield. Most suitable ph was 9 and temperature at 40ºc (1). **Secondly**, in the study of bacterial strain from degraded abattoir waste, three isolates were found namely yellow, white and orange colored bacteria. Amongst them, white colored colony was found to be more suitable for protease production. The morphological, cultural, biochemical and 16s rrna identified the bacterial strain. Physical and chemical parameters were optimized for maximum protease production and optimum temperature and ph was found to be 40ºc at ph 14. Glucose as a carbon source and yeast extract as a Nitrogen source further stimulated the production process giving maximum protease activity(4). **Lastly,** Screening and isolation of protease producing strains of bacteria were carried out from four different soil samples collected from various places in Bangalore. The isolates were positive on skim milk agar (1%) and thus are selected as protease producing strain. The organisms were tested for various biochemical tests, which lead to their identification. Optimal growth temperature and pH were found at 37ºC and 8.0 respectively. It was also optimized for carbon test and nitrogen test with optimal growth in dextrose and peptone respectively. Enzyme production was carried in 1 litre of optimized media in the fermenter at 37ºC for 48 hours at pH 8.0. Harvested protease product was purified by salt precipitation method. Finally the enzyme protease was purified by column chromatography. The protein was characterized using SDS-PAGE.(3) **Hence, these results showed that *Bacillus subtilis* under study is a good producer of protease which can be beneficial for industries in future needs for the protease production.**

[1] Student, M.Sc (Biotechnology), Christ College Jagdalpur, Bastar Distt. (CG), India

(II) Keywords

Abattoir waste, Bacillus subtitilis, protease, SDS-PAGE, extra cellular protease, optimization, beef extract, yeast extract, column chromatography, 16S RNA sequencing.

(III) Introduction

The enzymes are considered as "green chemicals" due to their ecofriendly nature. Also, they possess wide range of applications ranging from industrial sector to house-hold products(4). Enzymes are delicate protein molecules necessary for life. (3)Proteases, also termed proteinases or peptidases [1] are enzymes occurring everywhere in nature, be it inside or on the surface of living organisms such as plants, animals and microbes. These enzymes carry out proteolysis i.e. break down of proteins by hydrolysis of the peptide bond that exists between two amino acids of a polypeptide chain. Proteases are degradative enzymes which catalyze the total hydrolysis of proteins.(7). Protease are the single class of enzymes which occupy a pivotal position due to their wide applications in detergent, pharmaceutical, photography, leather, food and agricultural industries. An important biotechnological application of protease is in bioremediation processes (3). Microorganisms regarded as an important source of proteases because they can be obtained in large quantities using cultural techniques within a shortest possible and they produce a regular and abundant supply of the desired product.(5). Among various proteases, bacterial proteases are the most significant, compared to animal and fungal proteases (1). Proteases also known as peptidyl-peptide hydrolase and constitute 60–65% of the global enzyme market.. Proteases are commercially important enzymes having a wide range of applications in various industrial, biotechnological, medicinal and basic research fields (2). Though proteases are produced by a variety of bacteria such as Pseudomonas aeruginosa, Flavobacterium, Clostridium, Achromobacter, Thermo actinomyces and species belonging to Streptomyces, Bacillus sp. is the major source which secretes a variety of soluble extracellular enzymes (2).

A myriad of Bacillus species from many different exotic environments have been explored and exploited for alkaline protease production but most potential alkaline protease producing bacilli are strains of B. subtilis (5). B.subtilis are involved in the enzyme industries and produce a variety of intracellular and extracellular proteases (1). Bacterial alkaline proteases are characterized by their high activity at alkaline pH, e.g., pH 10, and their broad substrate specificity. Their optimal temperature is around 60°C. These properties of bacterial alkaline proteases make them suitable for use in the detergent industry (5). The demand for proteolytic enzymes having appropriate specificity and stability to pH, temperature, metal ions, surfactants and organic solvent is now rising, stimulating the

search for more new enzyme sources (4). Many researchers are moving towards exploring new sources for producing various enzymes. Conversion of wastes into useful biomass by microorganisms and their enzymes is a new trend, and new protease-producing microorganisms and perfected fermentation technology are needed to meet the ever-growing demand for this enzyme (4). Waste products of meat, poultry and fish processing industries can supply a large amount of protein rich material for bioconversion to recoverable products. Research has been done on many other related sources for protease production; a few to mention are, slaughter house soil, dairy industrial effluent, market waste, sewage waste (4). Further physicochemical parameters for maximum protease production from *B. subtilis* were optimized followed by purification and characterization studies (4). It is now known that single amino acid mutations in more than 50 human proteases result in hereditary/genetic diseases. Studies suggest that proteases are responsible for the complex processes involved in the normal physiology of the cell as well as in abnormal pathophysiological conditions. Their involvement in the life cycle of disease-causing organisms has led them to become a potential target for developing therapeutic agents against fatal diseases such as cancer and AIDS(7). In this study an attempt was made to study isolation and selection of bacterial strains that is potent producer of proteases and optimization of culture conditions required for enzyme production(3).

(IV) Literature and review

Microbes are the good sources of proteases. Since, proteases are enzymes of metabolic as well as commercial importance, there is vast literature on their biotechnological and biochemical aspect (ward, 1983; Fox et al, 1991 and Morihora and Oda, 1993). Bacillus species have been successfully used in degradation of proteinaceous wastes into useful biomass of proteases have also been demonstrated by many investigators. Bacillus species are the prolific producers of extracellular hydrolytic enzymes and in particular their production of protease (Godfrey and Reichet, 1983). Microbial proteases are among the most important hydrolytic enzymes that have been studied extensively since the advent of enzymology. There is renewed interest in the study of proteolytic enzymes, mainly due to the recognition that these enzymes not only play an important role in the cellular metabolic processes but have also gained considerable attention in the industrial community. These enzymes have become widely used in the detergent industry, since their introduction in 1914 as detergent additives (Guptal et.al.,2002).Micro organisms elaborate a large array of proteases, which are intra cellular and/or extra cellular. Intra cellular proteases are important for various cellular and metabolic processes, such as sporulation and differentiation, protein turnover, maturation of enzymes and hormones and maintenance of the cellular protein pool. Extra cellular proteases are important for the hydrolysis of proteins

in cell free environments and enable the cell to absorb and utilize hydrolytic products (Kalisz, 1998). At the same time, these extra cellular proteases have also been commercially exploited to assist protein degradation in various industrial processes (Outtrup and Boyce, 1990). Today proteases account for approximately 40% of the total enzyme sales in various industrial market sectors, such as detergent, food, pharmaceutical, leather, diagnostics, waste management and silver recovery (Gupta etal.,2002). Microbial proteases have been reviewed several times with emphasis on different aspects of proteases. Aunstrup (1980) focused on microbial selection and fermentation, where as Ward (1985) mainly dealt 21 with the sources of microbial proteases and their possible functional role in nature. Outtrap and Boyce (1990) focused on industrially important proteases, their applications and the role of molecular biology in protease research. The bioindustrial view points of microbial alkaline proteases from sources to cellular role, production, down stream processing, characterization and commercial application have also been reviewed (Anwar and Saleemuddin, 1998). An alkaline protease isolated from *Bacillus* species has optimum conditions of temperature and pH for activity (Krishna Suresh Babu etal., 2005).

This review article is based on the work of different researchers who worked on *Bacillus subtilis* for the optimized production of protease enzyme. The bacterial isolate was isolated from three different sources. The isolate from sea water showed optimum production of the enzyme at 37^0C and pH 9. The isolate from the abattoir waste had optimized production at 40ºC and pH 14. The isolate from the soil showed the optimized production of the enzyme at 40ºC and pH 8. The results clarified that the same species when taken from different areas and placed in a growing medium in lab, needed different parameter conditions to grow and survive. The protease assay proved that the sea water isolate produced the high amount of protease in comparison to other two sources. Hence, according to the result of the present study, sea water is the most suitable medium for production of proteases in high amount and their optimization in industries for future purposes. In view of the recent trend of developing environmentally friendly technologies, proteases are expected to have extensive applications in leather treatment and in several bioremediation measures. Proteases have gained commercial importance because of their potential applications in a wide range of industries including detergents, food and feed, leather, medical, diagnostic, pharmaceutical, waste management and recovery of silver from X-ray films. The applications of proteases in future may meet the need for a stable biocatalyst capable of withstanding harsh conditions of treatment. Because of the importance of microbial proteases in various industries, it is necessary to search for new enzymes at this time. The present study has taken up with a view to find out new microbial protease which is useful industrially.

(V) Objectives

1. The isolation of efficient protease producing bacteria,
2. Morphological and biochemical characterization of efficient bacterial isolate.
3. Optimization of physiological conditions for enhanced protease production.

(VII) Materials

1. Media preparation

(a) Zobell marine medium **(**HIMEDIA laboratories)

Ingredients	**Gms / Litre**
Yeast extract	1.000
Ferric citrate	0.100
Sodium chloride	19.450
Magnesium chloride	8.800
Sodium sulphate	3.240
Calcium chloride	1.800
Potassium chloride	0.550
Sodium bicarbonate	0.160
Potassium bromide	0.080
Strontium chloride	0.034
Boric acid	0.022
Sodium silicate	0.004
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Disodium phosphate	0.008
Agar	15.000
Final pH (at 25°C)	7.6±0.2

(b) Nutrient agar medium (HIMEDIA laboratories)

Ingredients	**Gms / Litre**
Sodium chloride	5.0
Beef extract	1.5
Yeast extract	1.5
Agar	15.0
Final pH (at 25°C)	7.4±0.2

(c) Gelatin agar medium (HIMEDIA laboratories)

Ingredients	Gms / Litre
Gelatin	30.000
Casein enzymic hydrolysate	10.000
Sodium chloride	10.000
Agar	15.000
Final pH (at 25°C)	7.2±0.2

(VIII) Materials and reagents

Gelatin yeast extract broth (Gelatin, glucose, yeast extract, potassium hydrogen phosphate), sucrose, fructose, cellulose, lactose, starch, xylose, glucose, dextrose, ammonium nitrate, ammonium chloride, ammonium sulphate, yeast extract, potassium nitrate, sodium nitrate, peptone, ferrous sulphate, potassium dihydrogen phosphate, magnesium phosphate, sodium chloride,tris-buffer, aqueous casein solution, trichloroacetic acid, sodium carbonate, folin phenol reagent, beef extract, yeast extract, urea, peptone, potassium chloride, magneese chloride, magnesium chloride, copper sulphate, magnesium sulphate, phosphate buffer, gram's stain.

(a) Glasswares used:

Test tubes, petri plates, beaker, glass rod, pipette, conical flask, culture tubes, cuvette.

(b) Instruments:

Centrifuge, spectrophotometer, incubator, automated DNA sequencer, shaker, fermenter, autoclave.

(c) Protocol:

(i) Sample collection: (1,2,3)

samples were collected from different locations as follows:

SN	Source	Location
1	Sea water	Cuddalore coast, Tamil Nadu (1)
2	Soil sample	Different places in Banglore. (3)
3	Abattoir waste	Local meat shop, Wadala, Mumbai. (4)

(ii) Isolation and screening of protease producing bacteria.(1, 2, 3)

The sample were isolated and cultured in lab in the following medium and the plates were incubated:

	Sea water	Soil sample	Abattoir waste
Medium	Zobell marine agar	Nutrient agar	Gelatin agar medium

	medium	medium	
Incubation	37ºC, 5 days	38ºC, 2 day	37ºC, 1 day

(iii) Identification of protease producing bacteria (1,2,3)

- The bacterial isolate was first identified by using Gram's staining method. (4)
- The morphological and physiological characteristic of bacterial isolate was studied according to Bergey's manual of determinative bacteriology. (4)
- Taxonomic identification based on above tests were carried out using 16s rRNA method. (4)

(iv) Optimization of selected isolates: (1,2,3)

	Sea water	**Soil sample**	**Abattoir waste**
temperature	20ºC, 30ºC, 40ºC,50 ºC, 60ºC, 70ºC, 80ºC	25ºC, 37 ºC, 40 ºC	20ºC, 30ºC, 40ºC, 50ºC, 60ºC, 70ºC, 80ºC
pH	3-12	6-9	3, 5, 7, 9, 10-14
Nitrogen sources	Yeast extract, beef extract, peptone, urea, ammonium chloride, sodium nitrate, ammonium sulphate	Beef extract, tryptone peptone	Ammonium nitrate, ammonium chloride, ammonium sulphate, yeast extract, potassium nitrate, sodium nitrate.
Carbon sources	Starch, glucose, maltose, lactose, xylose, fructose**.**	Dextrose, sucrose, lactose, maltose	Sucrose, fructose, glucose, cellulose, lactose, starch
Sodium chloride	1-10%	-	-
Metal ion	Cacl2, MnSO4, CuSO4, KCl, Mgcl2	-	-

Note: the strain *Bacillus subtilis* extracted from sea water existed in a marine habitat that is why protease production increased in presence of Nacl and decreased in its absence.

(v) Enzyme production:

- Sea water(1)

- Medium: glucose(0.5g), peptone(1g), ferrous sulphate(0.1g), magnesium sulphate(0.5g), sodium chloride(3g).
- 10 ml of medium was taken in a 100ml flask and sterilized in autoclave at 121ºC for 15 min and after cooling the flask was inoculated with overnight grown bacterial culture.
- The culture was then incubated at 37 ºC in shaker for 48 hrs.
- At the end of fermentation period, the culture medium was centrifuged at 5000 rpm for 15 min to obtain the supernatant.

- Soil sample(3)
  - Medium: dextrose(1%), peptone(0.5%), potassium dihydrogen phosphate(0.2%), magnesium sulphate(0.2%), casein(1%).
  - It was maintained at 37 ºC for 48hrs in a shaking incubator.
  - After inoculation, fermentation was carried out at 37 ºC at 200 rpm for 48 hrs.
  - At the end of each fermentation period, the whole culture broth was centrifuged at 10,000 rpm for 15 min to remove cellular debrises and clear supernatant was used as enzyme.
- Abattoir waste(2)
  - Medium: gelatin(1%), glucose(1%), yeast extract(0.2%), dipotasium hydrogen phosphate(0.3%).
  - It was incubated at 40 ºC upto 32hrs in an orbital shaker at 150 rpm.
  - The contents were then centrifuged at 5000 rpm at 4 ºC for 20 min .

(vi) Enzyme purification

The purification of the enzyme was done by ammonium sulphate precipitation method and column chromatography.(3, 2)

(vii) Protease assay

To study proteoloytic activity, supernatant was used as enzyme source. 1% casein in 0.1 M phosphate buffer and pH 7.0) was used as substrate. 1ml enzyme and substrate was incubated at 50 ºC for 60 minutes. To stop the reaction 3ml Trichloroacetic acid was used. One unit of protease activity was defined as the increase of 0.1 unit optical density at 1 hr incubation period. Then it was centrifuged at 5000 rpm for 15 minutes. From this, 0.5ml of supernatant was taken, to this 2.5ml of 0.5 M sodium carbonate was added, mixed well and incubated 20 minutes. Then it was added with 0.5ml of folin phenol reagent and the absorbance was read at 660 nm using Spectrophotometer (Bharat Pokhrel et al,2014.). The amount of protease produced was estimated and expressed in

microgram of tyrosine released under standard assay conditions. Based on the tyrosine released the protease activity (10).

(IX) Techniques used

(a) 16s rRNA gene sequencing method.
(b) Column chromatography.
(c) Polymerase Chain Reaction(PCR).
(d) SDS-PAGE
(e) Folin- lowry assay

(X) Result and discussion

In the present study, the protease producing strain *Bacillus subtilis* was isolated from three different sources viz., sea water, soil and abattoir waste.the morphological and biochemical tests revealed that it is a gram positive bacterium. The strains were provided with different growing mediums for optimized production of the protease.

(a) Isolation of bacterial culture

In the present study, the bacterial strains were isolated from different sources and plated on separate medium

Sea water	Soil sample	Abattoir waste.
Zobell marine agar medium	Nutrient agar medium	Gelatin agar plates

(b) Screening of protease producing organisms

In the present study, the isolated colonies were incubated at different parameters and then used for further studies.

Sea water	Soil sample	Abattoir waste.
37ºC, 5 days	35ºC, 2 day	37ºC, 1 day

(c) Effect of pH on protease production

In the present study the effect of pH by *Bacillus subtilis* revealed that optimum pH all the strains were different and they showed optimum protease production at different levels.

Sea water	Soil sample	Abattoir waste.
9	8	14

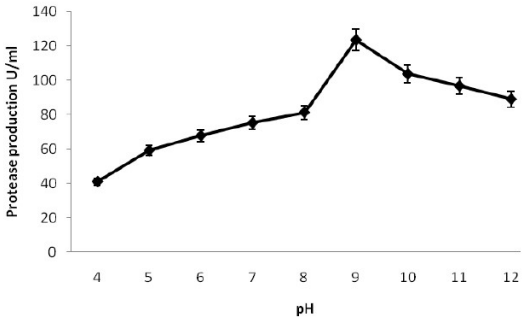


Figure 2. Effect of pH on protease production from *Bacillus subtilis*

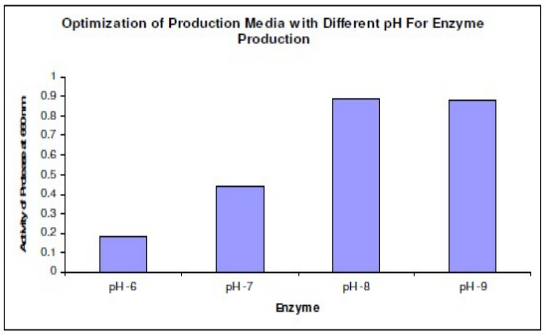
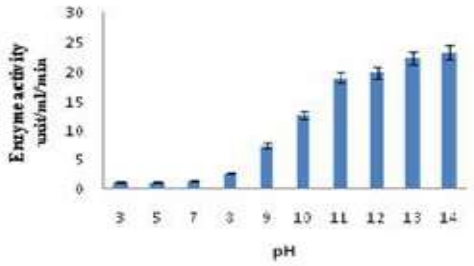


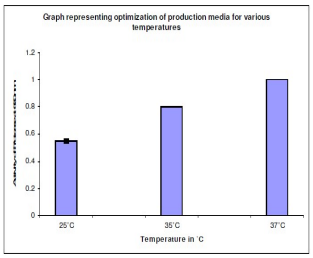
Figure 3. Optimization of production media for various pH



(d) Effect of temperature on enzyme production

In the present study, the production medium of all strains were supplied with various temperature levels according to which maximum yield by each strain was seen at that the following temperatures

Sea water	Soil sample	Abattoir waste.
37 ºC	40 ºC	40 ºC



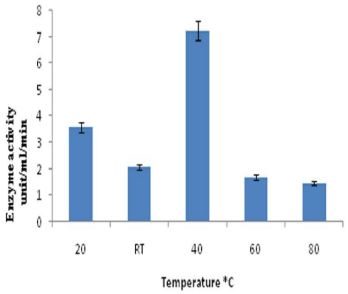


Fig 1: Optimization of production media for various temperatures

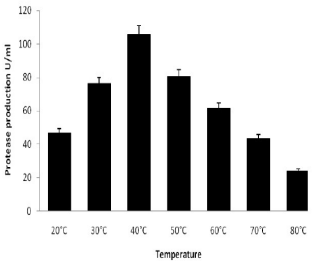
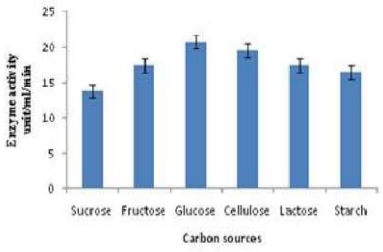


Figure 3. Effect of temperature on protease production from *Bacillus subtilis*

(e) Effect of carbon source on protease activity.

In the present study, many different carbon sources were used according for all the strains. Among which the following sources proved to be the most significant source for the given below strains

Sea water	Soil sample	Abattoir waste.
glucose	dextrose	glucose



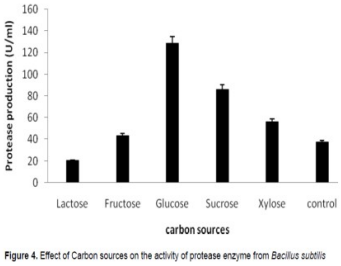


Figure 4. Effect of Carbon sources on the activity of protease enzyme from *Bacillus subtilis*

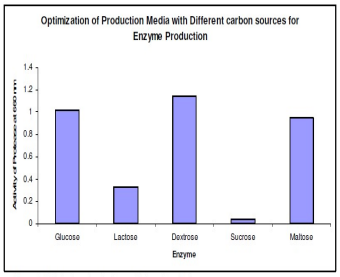


Figure 4. Optimization of production media for various Carbon sources

(f) Effect of Nitrogen source on protease activity-

In the present study, the supplementaed nitrogen source enhanced the production of enzyme. The highest production of protease were seen on the following sources-

Sea water	Soil sample	Abattoir waste.
Beef extract	peptone	Yeast extract

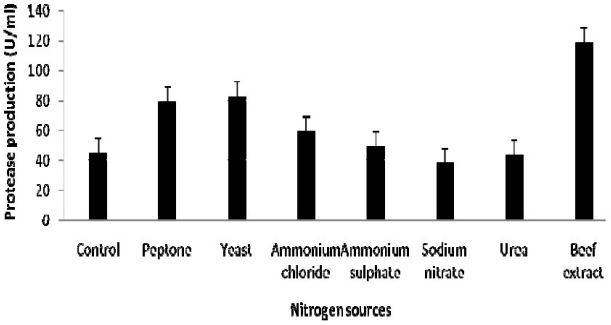


Figure 5. Effect of nitrogen sources on protease production from *Bacillus subtilis*

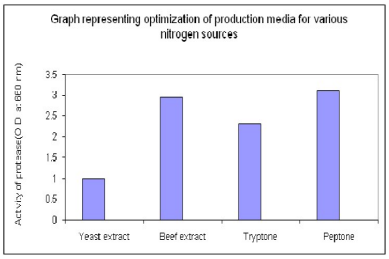
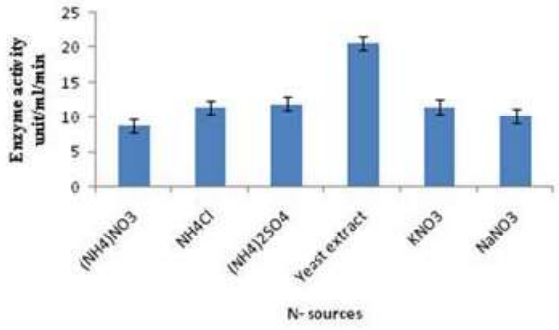


Figure 2: Optimization of production media for various nitrogen sources



(g) Effect of Nacl on protease activity

One of the isolate used in the present study was isolated from sea water and due to this reason the medium of this strain showed reduced protease production in the absence of sodium chloride whereas increased production in the presence of it. The maximum yied was seen at 7% Nacl.

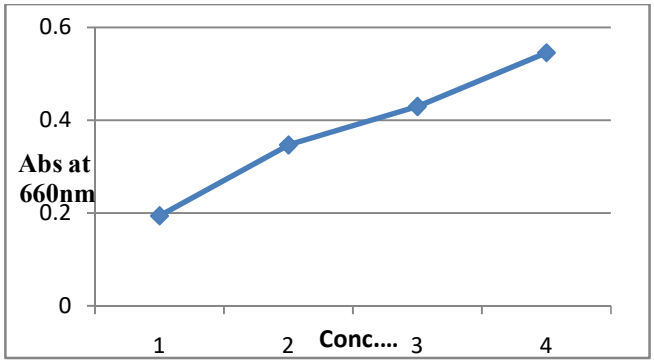
(h) Enzyme production and purification

The sample were inoculated in enzyme production medium and were undergone with fermentation period at certain parameters. Centrifugation was followed by the fermentation period. The supernatants were used as enzyme sources and were purified by column chromatography.

(i) Protease assay

Amount of protease produced according with the parameters optimally used. The results revealed that thebest and highest amount of protease was produced by strains isolated from sea water.

	Sea water
pH	123.5 U/ml
temperature	117.4 U/ml
Carbon	199.01 U/ml
Nitrogen	118.42 U/ml



(j) Applications-

Proteases have a large variety of applications, mainly in the detergent and food industries. In view of the recent trend of developing environmentally friendly technologies, proteases are envisaged to have extensive applications in leather treatment and in several bioremediation processes. The worldwide requirement for enzymes for individual applications varies considerably. Proteases are used extensively in the pharmaceutical industry for preparation of medicines such as ointments for debridement of wounds, etc. Proteases that are used in the food and detergent industries are prepared in bulk quantities and used as crude preparations, whereas those that are used in medicine are produced in small amounts but require extensive purification before they can be used.(9).Proteases execute a large variety of functions, extending from the cellular level to the organ and organism level, to produce cascade systems such as haemostasis and inflammation, which are responsible for the complex processes involved in the normal physiology of the cell as well as in abnormal pathophysiological conditions. Their involvement in the life cycle of disease- causing organisms has led them to become a potential target for developing therapeutic agents

against fatal diseases such as cancer and AIDS. Microbial proteases are increasingly used in treatment of various disorders namely cancer, inflammation, cardiovascular disorders, necrotic wounds etc. Proteases are used an immune–stimulatory agents. Increased antibiotic concentration at a target site when protease was concomitantly used with an antibiotic. Proteases are used extensively in the pharmaceutical industry for preparation of medicines such as ointments for debridement of wounds. It is also used in denture cleaners and as contact-lens enzyme cleaners. Proteases have a large variety of applications, mainly in the detergent and food industries. Proteases are envisaged to have extensive applications in leather treatment and in several bioremediation processes. Proteases that are used in the food and detergent industries are prepared in bulk quantities and used as crude preparations; whereas those that are used in medicine are produced in small amounts but require extensive purification before they can be used.(6)

The food industries are the major protease using industries. However, they have also found widespread application in laundry detergents. The thermo stability and their activity at high pH and the alleviation of pollution characteristic have made proteolytic enzymes an ideal candidate for laundry applications. Alkaline proteases are supplemented in different brands of detergents for use in home and commercial establishments. Enzymes have been added to laundry detergents since last 50 years to facilitate the release of proteinaceous material in stains such as those of milk and blood. The proteinaceous dirt coagulates on the fabric in the absence of proteinases as a result of washing condition. The enzyme removes not only the stain, such as blood, but also other materials including proteins from body secretion and food such as milk, egg, fish and meat. An ideal detergent enzyme should Be stable and active in the detergent solution and should have adequate temperature stability to be effective in a wide range of washing temperature. Usually the surgical instruments are washed or cleaned by sterilization or by using chemical steriliants. However, chemical steriliants cannot remove microbes that usually get trapped behind the bioburden that is encrusted on or within surgical instruments. However, the recent technologies include enzyme-containing formulations and zeolite based detergents. Of these, the enzyme detergents often referred to as “Green Chemicals” are proving useful in keeping a check on the environmental pollution and thus improving ecological situation. In leather industry, removal of hair and unwanted adhering subcutaneous layer by chemicals causes a problem. Hence the need for alternatives to sulphide dehairing is being sought. Tanners are hesitant to use the enzyme because of certain disadvantages in using them at commercial level for reasons of the stability of the enzyme at different environmental

conditions such as pH, temperature and duration consistent performance and the cost of production and application. The important factor in choosing an enzyme as a dehairing agent depends on the specificity of the enzyme used, which should not attack the collagenous matter. Numerous studies carried out from time to time to recover silver from photographic films as well as from x-ray films are patented. The silver recovery methods from these wastes includes: burning the films directly oxidation of metallic silver followed by electrolysis stripping the silver-gelatin layer using microbial enzymes specifically alkaline proteases and stripping the gelatin silver layer using different chemicals. Recovery of silver by burning the films creates environmental pollution and health hazards. On the other hand, enzyme from microbial source breaks the gelatin layer embedded with silver in films creating pollution free stripping. The amount of silver varies from 5-15 g/kg of film. Enzymatic method although slow is free from pollution and cost-effective too. The use of proteases in the food industry dates back to antiquity. They have been routinely used for various purposes such as cheese making, baking, preparation of soya hydrolysates, and meat tenderization. The major application of proteases in the dairy industry is in the manufacture of cheese.

Besides their industrial and medicinal applications, proteases play an important role in basic research. Their selective peptide bond cleavage is used in the elucidation of structure function relationship, in the synthesis of peptides, and in the sequencing of proteins. In essence, the wide specificity of the hydrolytic action of proteases finds an extensive application in the food, detergent, leather, and pharmaceutical industries, as well as in the structural elucidation of proteins, whereas their synthetic capacities are used for the synthesis of proteins.

(XI) Future scope

Proteases are a unique class of enzymes, since they are of immense physiological as well as commercial importance. They possess both degradative and synthetic properties. Since proteases are physiologically necessary, they occur ubiquitously in animals, plants, and microbes. However, microbes are a goldmine of proteases and represent the preferred source of enzymes in view of their rapid growth, limited space required for cultivation, and ready accessibility to genetic manipulation. Microbial proteases have been extensively used in the food, dairy and detergent industries since ancient times. There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer, malaria, and AIDS. Advances in genetic manipulation of microorganisms by SDM of the cloned gene opens new possibilities for the introduction of predesigned changes, resulting in the production of tailor-made proteases with novel and desirable properties. The development of recombinant rennin and its commercialization by

Pfizer and Genencor is an excellent example of the successful application of modern biology to biotechnology. The advent of techniques for rapid sequencing of cloned DNA has yielded an explosive increase in protease sequence information. Analysis of sequences for acidic, alkaline, and neutral proteases has provided new insights into the evolutionary relationships of proteases. Despite the systematic application of recombinant technology and protein engineering to alter the properties of proteases, it has not been possible to obtain microbial proteases hat are ideal for their biotechnological applications. Industrial applications of proteases have posed several problems and challenges for their further improvements. The biodiversity represents an invaluable resource for biotechnological innovations and plays an important role in the search for improved strains of microorganisms used in the industry. A recent trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or extremely acidic or alkaline habitats. The proteases isolated from extremophilic organisms are likely to mimic some of the unnatural properties of the enzymes that are desirable for their commercial applications. (9)

Exploitation of biodiversity to provide microorganisms that produce proteases well suited for their diverse applications is considered to be one of the most promising future alternatives. Introduction of extremophilic proteases into industrial processes is hampered by the difficulties encountered in growing the extremophiles as laboratory cultures. Revolutionary robotic approaches such as DNA shuffling are being developed to rationalize the use of enzymes from extremophiles. The existing knowledge about the structure-function relationship of proteases, coupled with gene-shuffling techniques, promises a fair chance of success, in the near future, in evolving proteases that were never made in nature and that would meet the requirements of the multitude of protease applications A century after the pioneering work of Louis Pasteur, the science of microbiology has reached its pinnacle. In a relatively short time, modern biotechnology has grown dramatically from a laboratory curiosity to a commercial activity. Advances in microbiology and biotechnology have created a favorable niche for the development of proteases and will continue to facilitate their applications to provide a sustainable environment for mankind and to improve the quality of human life.(9) Proteases are a unique class of enzymes, since they are of immense physiological as well as commercial importance. They possess both degradative and synthetic properties. Since proteases are physiologically necessary, they occur ubiquitously in animals, plants, and microbes. However, microbes are a goldmine of proteases and represent the preferred source of enzymes in view of their rapid growth, limited space required for cultivation, and ready accessibility to genetic manipulation. Microbial proteases have been extensively used in the food, dairy and detergent industries since ancient times. There is a renewed interest in proteases as targets for developing

therapeutic agents against relentlessly spreading fatal diseases such as cancer, malaria, and AIDS. The development of recombinant rennin and its commercialization by Pfizer and Genencor is an excellent example of the successful application of modern biology to biotechnology. Analysis of sequences for acidic, alkaline, and neutral proteases has provided new insights into the evolutionary relationships of proteases.(6)

Despite the systematic application of recombinant technology and protein engineering to alter the properties of proteases, it has not been possible to obtain microbial proteases that are ideal for their biotechnological applications. Industrial applications of proteases have posed several problems and challenges for their further improvements. The biodiversity represents an invaluable resource for biotechnological innovations and plays an important role in the search for improved strains of microorganisms used in the industry. A recent trend has involved conducting industrial reactions with enzymes reaped from exotic microorganisms that inhabit hot waters, freezing Arctic waters, saline waters, or extremely acidic or alkaline habitats. The proteases isolated from extremophilic organisms are likely to mimic some of the unnatural properties of the enzymes that are desirable for their commercial applications. The existing knowledge about the structure function relationship of proteases, coupled with gene-shuffling techniques, promises a fair chance of success, in the near future, in evolving proteases that were never made in nature and that would meet the requirements of the multitude of protease applications.(6)

(XII) Conclusion

The enzymes may serve as the model system and may pave the way for novel ways for eco-friendly industrial applications.(2). Proteases are industrially important enzymes with many applications especially in detergent industry (1). The present study shows the isolation of bacterial strain of *Bacillus subtilis* from sea water, soil and degraded abattoir waste. Successfully, optimized environmental factors and nutrient conditions yielded maximum protease production. But, among all the strains which gave optimized production of protease, strain isolated from sea water produced the enzyme in high amounts as compared to the others. Hence, it can be said that this enzyme can be produced in large scale from the microorganism *Bacillus subtilis* isolated from sea water (3). Based on the present study, it is concluded that *Bacillus subtilis* has wide scope for the industrial production of protease.

(XIII) References

1. N.S. Nisha J. Divakaran (2014) Optimization of alkaline protease production from *Bacillus subtilis* NS isolated from sea water Afr. J. Biotechnol. Vol. 13(16), pp. 1707-1713
2. Selvam Kandasamy1. Govarthanan Muthusamy2 . Senthilkumar Balakrishnan1,3. Senbagam Duraisamy4. Selvankumar Thangasamy5 . Kamala-Kannan Seralathan6. Sudhakar Chinnappan5 (2016) Optimization of protease production from surface-modified coffee pulp waste and corncobs using bacillus sp. By SSF 3 biotech 6:167
3. Das G, Prasad MP (2010) Isolation, purification and mass production of protease enzyme from Bacillus subtilis. Int Res J Microbiol 1:26–31
4. Pallavi Badhe1 Manasi Joshi2 Ravindra Adivarekar1 (2016) Optimized production of extracellular proteases by *Bacillus subtilis* from degraded abattoir waste J. BioSci. Biotechnol. 5(1):29-36
5. Bashir Sajo Mienda1,2,Adibah Yahya1, Ibrahim A Galadima1 and Mohd Shahir Shamsir1 (2014) An Overview of Microbial Proteases for Industrial Applications. RJPBCS 5(1) 388-395
6. Riddhi Sawant Saraswathy Nagendran* (2014) Protease: an enzyme with multiple industrial applications. World Journal of Pharmacy and Pharmaceutical Sciences. Volume 3, Issue 6, 568-579
7. Junaid furhan Sarika sharma*(2014) Microbial alkaline proteases: findings and applications Int.J.Inv.Pharm.Sci.,2(4);823-834
8. Abebe Bizuye, Abrham Sago, Genet Admasu, Hiwot Getachew, Paulos Kassa, Melese Amsaya(2014) Isolation, optimization and characterization of protease producing bacteria from soil and water in Gondar town, North West Ethiopia. Int. J. Bacteriol., Virol. Immunol. Vol. 1(3): pp 020-024
9. Mala b. Rao, aparna m. Tanksale, mohini s. Ghatge, and vasanti v. Deshpande* (1998) Molecular and Biotechnological Aspects of Microbial Proteases. Microbiology and molecular biology reviews . Vol. 62, No. 3. p. 597–635
10. Dalal Rupali; Screening and Isolation of Protease Producing Bacteria from Soil Collected from Different Areas of Burhanpur Region (MP) India, Int.J.Curr.Microbiol.App.Sci (2015) 4(8): 597-606
11. Virtual Amrita Laboratories Universalizing Educations (VALUE@ Amrita)
    - http://vlab.amrita.edu/?sub=2&brch=191&sim=341&cnt=1
    - http://vlab.amrita.edu/?sub=3&brch=186&sim=321&cnt=2
    - http://vlab.amrita.edu/?sub=3&brch=76&sim=1421&cnt=1
    - http://vlab.amrita.edu/?sub=3&brch=186&sim=319&cnt=1
    - http://vlab.amrita.edu/?sub=3&brch=64&sim=1087&cnt=1

जैमिनी अश्वमेध में भक्ति

डॉ0 विजयलक्ष्मी बाजपेयी[1]

सारांश

जैमिनी अश्वमेध में भक्ति तत्व श्रीमदभवत्गीता तथा भागवत पर आधारित है। जिस प्रकार गीता में भक्ति का कर्म ज्ञानमय रूप मिलता है उसी प्रकार उक्त खण्डकाव्य में भी कर्म प्रधानता है। गोपाल कवि ने अपने काव्य "जैमिनी अश्वमेध" में निर्गुण–सगुण का विवाद नहीं उठाया है, न ही वे किसी भक्ति संप्रदाय से संबंध रखते है। अवश्य ही, शैवमत तथा वैष्णव मत का सामंजस्य स्पष्ट दिखाई देता है ।

प्रस्तावना

भक्ति की परिभाषा–

संस्कृत की व्युत्पत्ति परिभाषा "भक्ति" शब्द 'भज' सेवायाय धातु से 'क्तिन' प्रत्यय लगाकर बनाया गया है। जिसका अर्थ है–भगवान का सेवा प्रकार। विषयभेद से भक्ति स्वरूप भेद और नाम भेद से भी हो जाता है। पूज्ययेष्वनु–रागोभक्तिः पूज्यवर्ग में भगवान का नाम भक्ति है।1

अन्य आर्ष ग्रंथ में उपलब्ध परिभाषा–

"यदत्र क्रियते कर्म
भगवत् परितोषणम्।
ज्ञानं पत्र दधीनम् हो
भक्ति योग समान्वितम्"

आचार्य रामचन्द्र के अनुसार –

"श्रद्धा और प्रेम के योग का नाम भक्ति है। जब पूज्य भाव के साथ श्रद्धा भाजन के सामीप्यभाव की प्रवृत्ति हो, कई रूपों के साक्षात्कार की भावना हो, तब हृदय में भक्ति का प्रादुर्भाव समझना चाहिए।"

भक्ति के प्रकार–

(1) गीता में उपलब्ध प्रकार–

"चतुर्विद्या भजन्ते मा जनाः सुकृतिऽनार्जनुः आर्तो जिज्ञासुरार्थी ज्ञानी च भरतर्षभ"

(2) डॉ0 हजारी प्रसाद द्विवेदी के अनुसार –

(क) रागात्मिका

(ख) रागानुगा

(ग) गौणी/वैधी–इसके अर्न्तगत नवधा भक्ति का उल्लेख तुलसीदास कृत 'रामचरितमानस' शबरी को भक्ति योग का संदेश श्री रामचंद्रजी देते हैं।

[1] सहायक प्राध्यापक, हिन्दी विभाग, क्राईस्ट महाविद्यालय, जगदलपुर, जिला–बस्तर (छ0ग0)

"सत्संग कथा, श्रवण, पादसेवनम, गुणगान, मंत्र जाप एवं भजन, शीलस्वभाव, संत की महत्ता, संतोषी, सीधा स्वभाव" में नौ प्रकार की भक्ति मानी गई है।"

(3) भावों की दृष्टि से–

डॉ. हजारी प्रसाद द्विवेदी ने भावों के अनुसार पाँच प्रकार की भक्ति को स्वीकार किया है। माधुर्य, दास्य, सख्य, वात्सल्य, शान्त।

गोपाल मिश्र के "जैमिनी अश्वमेध" खण्डकावय में अर्जुन, भीम, युधिष्ठिर आदि भक्त साधरणतः आर्त रूप में प्रतीत होते हैं। यज्ञ का महान कार्य श्रीकृष्ण के बिना संभव नहीं था इसलिए पाण्डव श्रीकृष्ण को एकमात्र संबल मानते हैं।"

एक भरोसो होत है, नहिं सूझै कछु और,
होत सहायक कृष्ण हैं, संकट में सब और।

"यद्यपि पाण्डव संकट या कठिनाई के समय ही श्रीकृष्ण को स्मरण करते साधरणतः दिखाई पड़ते हैं किन्तु यह मान लेना उचित नहीं है कि अनापत्ति काल में उनकी श्रीकृष्ण भक्ति कम पड़ जाती है।"

उक्त खण्डकाव्य में अर्थार्थी भक्ति नहीं दिखाई देती। "जैमिनी अश्वमेध" में ऐसा कोई पात्र नहीं है जो प्रतिक्षण भावावेश में रहता हो, वह कभी हंसता हो या कभी उच्च स्वर में प्रलाप करता हो।

रागानुगा भक्ति का रूप नहीं दिखाई पड़ता। वैधी, भक्ति के अर्न्तगत नवधा भक्ति का उल्लेख "जैमिनी अश्वमेध" में मिलता है।

(1) श्रवण– भगवान की लीला यज्ञ आदि का सुनना और सुनाना ही श्रवण भक्ति है। गोपाल कवि ने इसे भक्ति और ज्ञान दायिका के रूप में स्वीकार किया है।

कृष्णदेव भूप सों अनेक भांतियों कहे।
अश्वमेध पुज्य ते न पाप देह में रहे।
है कथा रसाल में मनुष्य देवता सुने।
पुण्य कोन पारावार पाप शीश को धुने।

(2) कीर्तन–भगवान के नाम, लीला, यज्ञ आदि का गान कीर्तन कहलाता है। भक्त पर जिन गुणों का संस्कार प्रबल होता है उन्हीं का आरोप वह परमात्मा में कर लेता है।

जोरि जोरि हाय सकल राजा भये ठाढ़े
अस्तुति सब करत कृष्ण हृदय बाढ़े।
जै–जै प्रभुदीन बंधु अधम सब उधारे।
कोटिन अपराध कटि पातक ते तारे।"

(3) स्मरण–गोपाल कवि ने हरि–भजन को ही भक्ति कहा है। 'मनसावाचाकर्मणा' नाम स्मरण करना चाहिए। प्रस्तुत खण्डकाव्य में अर्जुन कठिनाइयों के समय श्रीकृष्ण स्मरण करते हैं।

"जो जिहि भाँति जहां सुमिरै,

हरि सो तिहि भाँति तहां उठि धाये।"

(4) पादसेवनम्– श्रीमद भगवत् के अनुसार श्रीहरि के चरण अमित महिमाशाली हैं । ये असंभव घटना को संभव कर सकने में समर्थ हैं।

चरण पखारि नर नाघ लै लगाये माथ,
कीन्हे मो सनाथ यदुनाथ सुख पाइके।

(5) वंदन – साष्टांग प्रणाम भक्त के आत्मसमर्पण और दास्यभाव का परिचायक है। गोपाल कवि ने भी वंदन भक्ति का गान किया है।

वंदन करि कृष्ण चरण शल्य सुयश गाये।
पूरन–मन काम सुफल नैन दरश पाये।।
अद्भुत अवगति अपार कौन पार पाये।
कीन्हे ध्रुव अटल आपु कीरति श्रुति गाये।।

(6) अर्चन – श्रद्धा सहित भगवान की उपासना ही अर्चन भक्ति है। गीता में अर्चन भक्ति का विस्तार वर्णन किया गया है। श्रीकृष्ण कहते है–श्रेष्ठता के अभिमान का अभाव, दम्भाचरण का अभाव, क्षमाभाव, श्रद्धासहित गुरू की सेवा तथा बाहर भीतर की शुद्धि से भगवान की सेवा की जाती है।

जैमिनी अश्वमेध में युधिष्ठिर श्रीकृष्ण की अर्चना करते हैं–

अस्तुति करत युधिष्ठिर राय,
प्रभुबिन हीन कौन सहाय,
सब मेरा पूज्यो मन काम,
अब प्रभु पाइ धारिये धाम।।

(7) दास्यभाव–इसके अन्तर्गत भक्त अपनी हीनता का परिचय देता है। प्रस्तुत खण्डकाव्य में युधिष्ठिर दास्य भाव से भक्ति का परिचय देते हैं। युधिष्ठिर की भक्ति गंभीर है–

प्रभु दीनबंधु दयाल दीनानाथ केशव श्रीमते।
करण कारण तरण तारण चरण पंकज मोमते।
तुम यज्ञ पुरूष अनादि बपु धरि यज्ञ पूरण है किये।
सब आप अर्चन के सुभावहिं हमहि जय कीरति दिये।

(8) सख्य भाव– पुष्टिमार्ग में सख्य भाव की पुष्टि का विशेष महत्त है। सख्य भाव में भक्त भगवान की प्रत्येक लीला में भाग लेता है। उसे ईश्वर के प्रति अनन्य मित्र भाव हो जाता है। "जैमिनी अश्मेध" में अलौकिक व्यक्ति की लौकिक लीला को मित्र रूप में चित्रित किया गया है। अर्जुन तथा भीम की भक्ति सख्य भाव से युक्त हैं।

(9) आत्मनिवेदन– यह नवधा भक्ति की अंतिम विधि है। इसे शरणागति भी कहते हैं। श्रीमदभगवत् गीता में भक्ति रस की पाँच धारायें मानी गई है–

शान्त, दास्य, सख्य, वात्सल्य, मधुर।

इस विधि में भक्त के लिए न तो कुछ विचारणीय रह जाता है और न करणीय। भक्त अपना सर्वस्व ही हरि को अर्पण करके निर्द्वन्द हो जाता है। शरणागत को अनेक भूमिकाओं से गुजरना पड़ता है।

भक्ति के अनूकूल आचरण का संकल्प, प्रतिकूल आचरण का वर्जन, परमात्मा रक्षा करेगा ऐसा विश्वास, रक्षा या सहायता की याचना, आत्मसमर्पण आदि।

गोपाल कवि के शरणागत भाव में समस्त भूमिकायें मिलती हैं। दास्यभाव में आत्मसमर्पण की भावना सन्निहित है । अर्जुन श्रीकृष्ण के प्रति समर्पित हैं। उनमें समर्पण–भाव कूट–कूट कर निहित है –

अर्जुन गहे चरण प्रभु गाढ़े, लोचन नीर तरंगनि बाढ़े,
अस्तुति भाँति अनेक उघारे, संकट में पति राखन होय।

इस उद्धरण में एक साथ अर्चन, समर्पण की भावना दिखाई देती है–

अनुजहिं सहित युधिष्ठिर राजा कृष्ण चरण गहे।
तब प्रभु दीन दयाल दया करि पाप सिंधु काढ़े।

उपर्युक्त विवेचन गीता के अनुसार प्रकार तथा महावीर प्रसाद द्विवेदी जी के अनुसार भक्ति के प्रकार की तुलना "जैमिनी अश्वमेध" से की गई है।

मधुरा भक्ति में केवल दाम्पत्य भाव होता है । "जैमिनी अश्वमेध" वीर प्रधान होने के कारण माधुर्य भाव की झलक मात्र दिखाई देती है। माधुर्य भाव का पूर्ण परिपाक नहीं हुआ है।

यहां पर द्रौपदी कहती हैं –

करैं मया कासों अधिकाय।
यह कछु भेद कहौ समुझाय
सुनि रूकमिनी सुकुची मन ऐन।
सतिभामा को दीन्हें सैन।

दास्य भाव बहुत व्यापक है। अर्जुन सुधन्वा युद्ध में सुधन्वा अर्जुन को विरथ कर देता है, ऐसी स्थिति में वे श्रीकृष्ण का स्मरण करते है–

बंदि कृष्ण चरणारविंद अर्जुन शर मारे,
तबहिं सुधन्वा वीर खण्ड–खण्ड करि डारे।

युधिष्ठिर दास्य भाव से युक्त हैं। प्रस्तुत खण्डकाव्य में अर्जुन–भीम की भक्ति सख्य भाव से युक्त हैं। किंचित दास्य भाव भी है।

"जैमिनी अश्वामेध" में वात्सल्य भाव किसी पात्र में दिखाई नहीं पड़ता।

शांति भाव में भक्त सभी सांसारिक मायामोह को त्याग कर शांत मन से भगवत्–भक्ति में रम जाता है।

परमारथ परमातम ज्ञान।
ते परलोक सुधारे न्यान।।
हरि मंदिर रचि बाग अरू ताल।
सात्विक करत जे कर्म,
जिनके अमित बखाने धर्म।।

भक्ति की अन्य विशेषतायें –

(1) कर्म व भक्ति का योग–

गोपाल कवि ने भक्ति व कर्म में सामंजस्य स्थापित किया है। अकर्मण्य व्यक्ति अंधविश्वासों और मिथ्याडम्बरों में फंसकर ढोंगी बन जाता है तथा प्रत्येक के अन्धानुकरण में लग जाता है जिससे बहुत अनिष्ट होता है। ज्ञान विहित की भांति कर्म विहित व्यक्ति का दान, तीर्थाटन एवं अन्य साधनायें सभी निष्फल हो जाती है। बिना कर्म के भगवत् भक्ति का सही रूप समझ में नहीं आ सकता। इससे सिद्ध होता है कि भक्ति भक्त को अकर्मण्य नहीं बनाती। गीता में श्रीकृष्ण अर्जुन को कर्म करने में सततरत् होने की प्रेरणा देते हैं।

"कर्मण्येवाधिकारस्ते मा फलेषु कदाचन्।
मा कर्मफलहेतुर्भूर्मा ते संगोऽस्त्वकर्मणि।।"

कर्म के फल की आशा किये बिना ही अपने कार्य में संलग्न होना भक्त की अनिवार्य एवं प्रथम स्थिति है। धर्मराज युधिष्ठिर अपने गोत्र पाप के उद्धार के परिणाम स्वरूप अश्वमेध यज्ञ की योजना बनाते हैं।

यद्यपि उनके पास विशाल सैन्य वाहिनी का अभाव है किन्तु श्रीकृष्ण उन्हें कर्म एवं भक्ति का मार्ग दर्शन कराते हुये कर्मरत होने की ओर संकेत करते हैं।

"जे जे उत्तम कर्म है, करत सयाने लोई।
वेद आचरण वर्ण कोई कियो कुतारथ होई।।

इस उदाहरण से स्पष्ट होता है कि उच्च कर्म बुद्धिमान गण ही कर सकते हैं।

"प्रबल धनुषधारी कौन ताको कौन चितारे।
हृदय भगति भावे नाम मेरो उघारे।।"

"जैमिनी अश्वमेध के सारे भक्त पात्र भक्ति के प्रति जितने उन्मुख हैं उतने ही मानव कर्तव्यों के प्रति सचेत हैं। यदि ऐसा न होता तो श्रीकृष्ण को अपने भक्तों के साथ युद्ध करने का प्रसंग न आता ।"

(2) अनन्यता–

श्रीमदभगवत गीता के अनुसार कुसंग का त्याग और अनन्यता की स्थिति जीवन में आते ही भक्त का पूरा भार भगवान पर आ पड़ता है। अतः इस ओर प्रयास आवश्यक है।

जैमिनी अश्वमेध के सभी पात्र केवल श्रीकृष्ण पर भरोसा रखते हैं अन्य किसी पर नहीं। सुधन्वा को अनेक कष्टों का सामना करना पड़ा किन्तु वे श्रीकृष्ण के अतिरिक्त किसी अन्य से आशा नहीं रखते हैं। "जैमिनी अश्वमेध" में गोपाल कवि ने यथास्थान अनन्यता का भाव प्रदर्शित किया है।

संकट नाशन नाम गोपाल सदा सब ठौर सहायक हैं।
शरणागत आरत पंतनि के बलवंतनि केवल धायक हैं।

"जैमिनी अश्वमेध" के भक्त के आचरण पर कवि लिखता है–वह संन्यासी होता है । उसकी सारी क्रियायें हरि के निमित्त होती हैं। अपने इष्ट पर उसका अनन्य विश्वास होता है। योग यज्ञादि पद उसका कोई आकर्षण नहीं रहता। उसमें दैन्य भावना होती है। अपने समस्त कर्मों का फल वह उपास्थ के चरणों में अर्पित कर देता है।"

निष्कर्ष –

इस तरह उपर्युक्त विवेचनोपरान्त यह ज्ञात होता है कि गोपाल कवि की भक्ति में किसी प्रकार की कमी महसूस नहीं होती। उन्हीं ने भक्ति परक सभी तथ्यों को "जैमिनी अश्वमेध" में प्रस्तुत किया है। यद्यपि गोपाल मिश्र में सूर, तुलसी जैसी उत्कृष्टता नहीं दिखाई पड़ती, किन्तु वे भक्त थे। "जैमिनी अश्वमेध" में भक्ति तत्व श्रीमदभगवत् गीता तथा भागवत् पर आधारित है। जिस प्रकार गीता में भक्ति का कर्म ज्ञानमय रूप मिलता है उसी प्रकार उक्त खण्डकाव्य में भी कर्म की प्रधानता है। गोपाल कवि ने "जैमिनी अश्वमेध" में निर्गुण–सगुण का विवाद नहीं उठाया है न ही वे किसी भक्ति संप्रदाय से संबंध रखते हैं।

अन्ततः हम कह सकते हैं कि "जैमिनी अश्वमेध" में भक्ति न पुष्टिमार्गी है न ही उसमें विशिष्टाद्वैतवाद की मान्यता ही परिलक्षित होती है। समूचे खण्डकाव्य में श्रीमदभगवत् गीता तथा भागवत की भक्ति परम्परा का आधार लिया गया है।

सहायक संदर्भग्रंथो की सूची –

(1) बाजपेयी डॉ नारायण प्रसाद–भक्ति काव्य की दार्शनिक चेतना पृ.9
(2) श्रीमदभगवतगीता, अध्याय 7, पृ.129
(3) शुक्ल, आचार्य रामचंद (प्रतिनिबंध) (श्रद्धा और भक्ति) पृ.39
(4) भागवत–प्रथम स्कंध, पंचम अध्याय 35 वां श्लोक, पृ.57, (गीता प्रेस गोरखपुर)
(5) डॉ चंदेले, शेषनारायण–"गोपाल मिश्र की काव्य साधना, पृ.44
(6) श्री बाल्मीकि रामायण–अरण्यकाण्ड, पृ.543–544
(7) मिश्र, गोपाल–जैमिनी अश्वमेध–अध्याय 1
(9) श्रीमदभगवतगीता–अध्याय 2 श्लोक 47 पृ.16

(10) मिश्र, गोपाल–जैमिनी अश्वमेध" अध्याय 18
(11) मिश्र–गोपाल–जैमिनी अश्वमेध"अध्याय 19
(12) डॉ. चंदेले, शेषनारायण– 'गोपाल मिश्र की काव्य साधना पृ.43
(13) मिश्र, गोपाल–"जैमिनी अश्वमेध" अध्याय 11
(14) डॉ. चंदेले, शेषनारायण–गोपाल मिश्र की काव्य साधना–पृ.47

Published by

CHRIST COLLEGE

GEEDAM ROAD, JAGDALPUR, DIST.-BASTAR (C.G.) 494001, INDIA

Email : christcollegejagdalpur@gmail.com