

ST. ALOYSIUS' COLLEGE

AUTONOMOUS JABALPUR- 482001 MADHYA PRADESH, INDIA

CRITERION-1



CURRICULAR ASPECTS

Key Indicator – 1.3

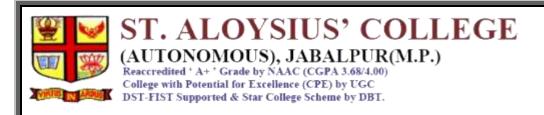
Curriculum Enrichment

Metric No.: 1.3.3

Percentage of programmes that have components of Field projects / Research projects / Internships during the last five years.

Document Name

Sample Evaluated Project report/Field work Report submitted by the students



SAMPLE PROJECT REPORTS 2021-22

FACULTY OF COMMERCE

Page 1

STUDY ON E-BANKING







Field Project Work Report

प्रोजेक्ट (परियोजना) कार्य

A STUDY ON E - BANKING

(Title of Field Project Work)

For the partial fulfillment of B.A./ B.Sc./ B. Com./ BBA/ BCA Degree बी.ए./बी.एस.सी./बी.कॉम./बी.बी.ए./बी. सी.ए./ की डिग्री की आंशिक प्रतिपूर्ति हेतु

Session: 2021-22



St. Aloysius' College (Autonomous)

Reaccredited 'A+' by NAAC (CGPA 3.68/4.00)
College with Potential for Excellence by UGC
DST-FIST Supported & Star College Scheme by DBT
Jabalpur, Madhya Pradesh, India

Field Project Work Report परियोजना कार्य रिपोर्ट ON E-BANKING (Title of Field Project Work) For the partial fulfillment of B.A./ B.Sc./ B. Com./ BBA/ BCA Degree बी.ए. / बी.एस.सी. / बी.कॉम. / बी.बी.ए. / बी.सी.ए. / की डिग्री की आंशिक प्रतिपूर्ति Session: 2021-22. MEGHNA PATEL Name of student/ Students (छात्र का नाम) B: Com (Hons.) । Year. Class (कक्षा) 1210052 Roll No (अनुक्रमांक) HDFC BANK VIJAYNA GAR Name of the organization, where the work was completed (संस्था का नाम जहाँ कार्य पूर्ण किया गया) DR. KOMAL RAWAT. Name of Supervisor (पर्यवेक्षक का नाम)



St. Aloysius College

(Autonomous)

Reaccredited 'A+' by NAAC (CGPA – 3.68/4.00)
College with Potential for Excellence by UGC,
DST FIST Supported & Star College Scheme by DBT
Jabalpur (M.P.) India

Institute information and consent letter for Field Project Work परियोजना कार्य के प्रशिक्षण हेतु संस्थान की जानकारी एवं सहमति पत्र

1.	Name of the Institute/Trainer/Business and Registration (संस्थानव्यवसाय का /प्रशिक्षक/
	नाम एवं पंजीकरण):
	Nature of the Institution (Private Government / Semi-Government / Other)
2.	
	संस्था का स्वरूप (अन्य/ अर्धशासकीय/शासकीय/ निजी) रिर्भारवीट
3.	Name of the area of expertise of the Institute in which work is done (संस्थान के
	मार्गदर्शक क्षेत्र का नाम, जिसमे कार्य किया जाता है(
	Banking
4.	Number of persons holding various posts/working under the institute (संस्थान के अंतर्गत
	विभिन्न पदों / कार्य करने वाले व्यक्तियों की संख्या) Confidential to Institution
5.	Maximum number of students, which can be trained by the institution (अपेक्षित
	अधिकतम विद्यार्थी संख्या जिनको संस्थान प्रशिक्षण दे सकता है)
	tuo (2)
	Scope of employment in organized / unorganized sectors after training from the
6.	Scope of employment in organized / unorganized sectors after training from and institution) संस्था से प्रशिक्षण के उपरांत संगठित असंगठित क/्षेत्रों में रोजगार की
	सम्भावना):(i)
)i)ii)
)iii)
7.	Other Specific Information (अन्य विशेष जानकारी)
	College College
Con	sent is given to provide Field Project work to the students of St. Aloysius Collage
(Au	tonomous), Jabalpur by the institution. हारा ,संत अलॉयासियस महाविद्यालय जबलपुर ,(स्वशासी) के विद्यार्थियों को प्रशिक्षण प्रदान करने
संस्थ	त द्वारा ,सत अलायासियस महानिवासिय अपने उत्तर के ति है।
की र	तहमति प्रदान की जाती है । Seal and Signature कि न ् रुम् हुन्मा र)
	Name of the Head of the Institution / Authorized Person
	Name of the Head of the Institution / त्रुवार्गिक्ट व्यक्ति का नाम/)
	(सस्या प्रमुख्याव्याव्याव्याव्याव्याव्याव्याव्याव्याव

Field Project Feedback Form

To be filled by the head of the
To be filled by the head of the institution / authorized officer / guide of the concerned external organization (if any)
भेरविभिने बीहा सम्बा (यदि कोई को) के न
सम्बंधित बाह्य संस्था (यदि कोई हो) के संस्था प्रमुख /अधिकृत अधिकारी /मार्गदर्शक द्वारा भरा जाये Name of trainee student (प्रशिक्ष विद्यार्थी का नाम): Name of College (सहाविद्यारक
Name of College (महाविद्यालय का नाम):
Class 事計: P. Com (Hone) 18- Year
Section & Roll No. (वर्ग तथा अनुक्रमांक) । 2) 0052

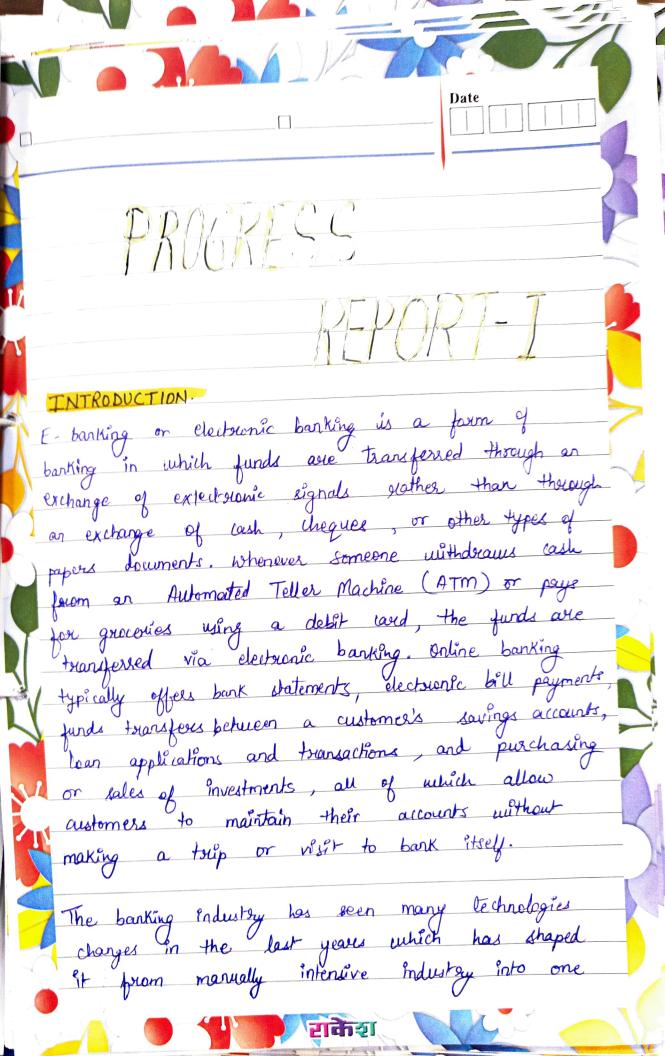
S.No	Day of F		
स्क्र	Base of Evaluation	Given	Remark
,,,,,	मुल्यांकन का आधार	Grade	टिप्पणी
		प्रदत्त श्रेणी	
		(A/B/C) #	
1.	Student's regular attendance		
	विद्यार्थी की नियमित उपस्थिति	1 1	
2.	Theoretical knowledge acquired by the student		
	विद्यार्था द्वारा प्राप्त मेद्धान्तिक ज्ञान	Ku	
3.	Skills and practical knowledge acquired by the student during the		
	tenure		
	कार्याविध में विद्यार्थी द्वारा अर्जित कौंशल व्यवहारिक ज्ञान	1 B	
4.	Student's interest and seriousness towards the work,	1	
	कार्य के प्रति विद्यार्थी की रूचि गंभीरता,		
5.	Student's attitude and behavior during the learning period		
	कार्यावधि में विद्यार्थी का मीखने के प्रति रवैया एवं व्यवहार	I A	
6.	Co-ordination with colleagues and other members and ability to		
	work in teams		
	सहकर्मियों व अन्य सदस्यों के साथ सामंजस्यसमूह में कार्य करने की ,	0	
	छमना		
7.	Overall grade of the student		
	विद्यार्थी की समग्र श्रेणी	\bowtie	

Grade (श्रेणी) : A-> Excellent (उत्कृष्ट) ,B-> Good (अच्छा) C-> Normal (सामान्य)

Date (दिनांक): 5/ 4/22 ... Place (स्थान) : . Tchalpur: ..

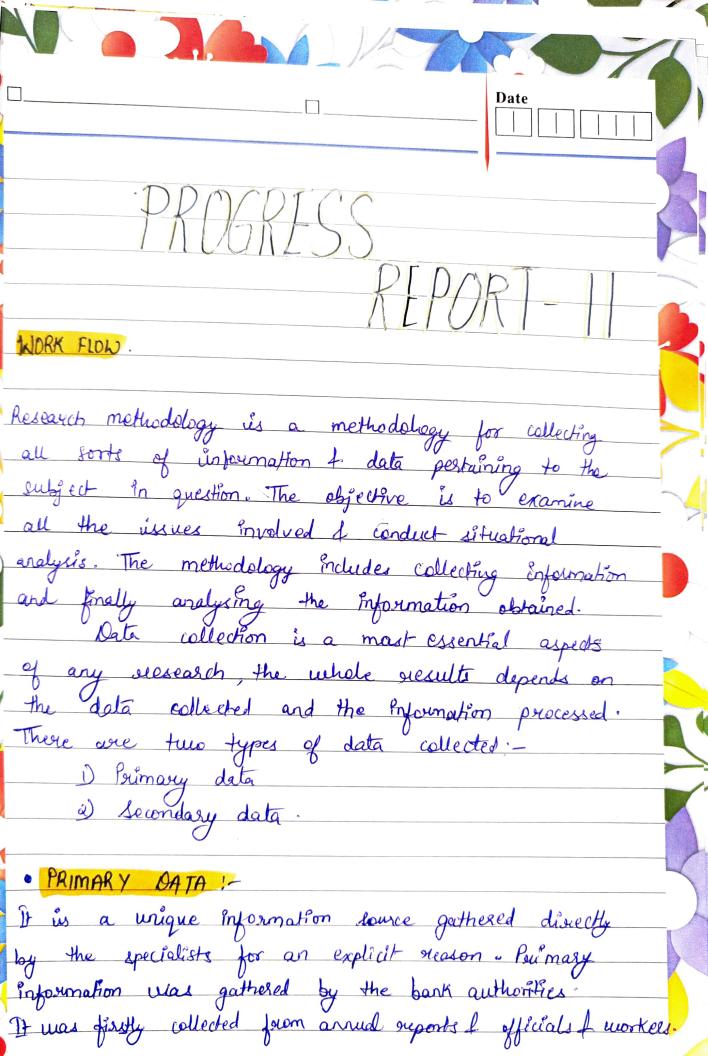
Name of Authorized Person (अधिकृत व्यक्ति के हस्ताक्षर)

Name and Seal (नाम 🐔

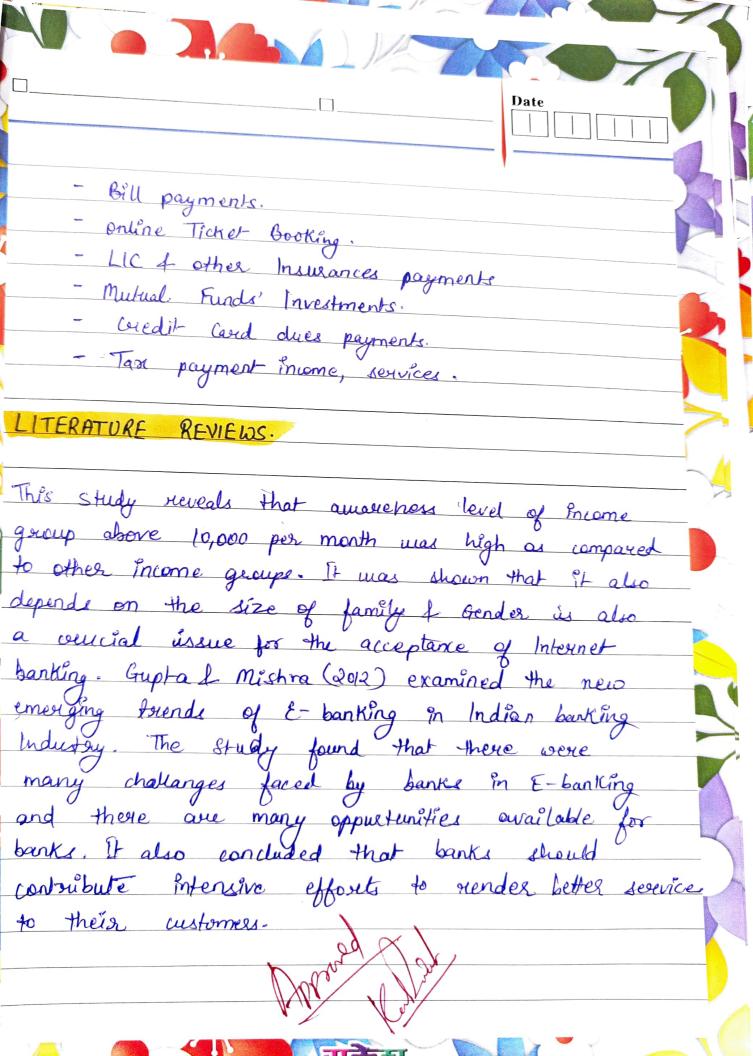


that is technologically dependent. SCOPE. The totaditional mode of banking is manual; it is paper based. It is now no longer acceptable in the changing economy. The banking sector if it is to surine in the 21st Century, shall have to change the entire banking structure. It shall have to provide better, economical and quick monetary services through the use of advance electronic technology. The customer of the banking are fast becoming computer literature. The needs of customers have also changed. They require the sources of bank at home, in offices, 34/7 a day, anywhere, anytime of anyhow. The banks are quite alive to the changing needs of the customers. The scope of E-banking is now fast expanding in the developed and also under-developed countries of I the woorld. THE WORKPLACE. This project support has been completed out HDFC Bank. The Housing Finance exporation Limited (HDFC) the tagline 'we understand your world', It is private sector bank. The bank is situated

Vijaynagar, Jabalpur. It is well known as the premier executive housing finance company in India. All branches are linked to each other through an online real time basis. PURPOSE AND RELEVANCE. This study is basically beised on primary & recondary data, this includes understanding E-banking in India introduction, scape of background of E-burking, also the Ennovations that took place in E-banking and various other details to E-banking. These objectives were achieved during the short period of survey at bank. Through this report we come across the Impostance and necessity of e-banking in the coming future as It is time laving and easy to understand. It is also noticed that the amareness and use of e-banking is growing with a great pace amongst the new age group and to the customer as "It has many types of C-Servicer like bill payments and doing any kind of transactions.



· SECONDARY PATA:-It is gathered from other than primary sources, who already gethered the information. Secondary data was gathered from published materials, books and websites and records. DETAILS OF INFORMATION COLLECTED. · Services offered by HDFC online Net-banking with HDEC Bank Not banking, users can do the following tolansactions via their desktop. · HDFC Bank customers can transfer funds between their own accounts or to another account of HOFC bank or different bank of the third party using as internet banking facility. with internet banking features, customers can also request for assues of Dennet Draft & Cheque book, the opening of new accounts and closure of Loan Accounts · others services offered by HDFC Bank are g-



PROGRESS ANALYSIS OF INFORMATION. During the survey at HOFC bank the information collected was of 2-banking and various other securices offered by banks through not banking. The method used to analyze the data is descerptive and also primary & secondary date in also used to analyze. After the completion of survey and Standing the results obtained are these :and the innovations that took place, also the services that are provided through ner bounting and their procedures and steps to get the results. Interenet banking helped impreoring the perofite and reduced the physical puesence of the person itself in the banks. It is also considered the cheapest delivery channel as It is free of cost and charges. It has helped in cutting costs, improving innovations, maintaining e-business

scourfces. Also, it is analyzed that mostly customers thank the transactions history and their account balances, so E-banking has eased the physical presence and time of customer at the bank to get such simple information about their accounts. E-banking pravides faster delivery of seowices to under range of the automers. Although these are also challanges, even after the challanges the completion of the survey and study gives are are superficilly results:

CHALLENGES.

Nowadays' electronic banking is a norm rather than an exception for the banks. But in spile of numerous assistance for the customes to make banking vary and convinent, there are many challanges which customers are failing in the adoption of E-banking. Some are stated below:—

1. Customers siefuse to adapt E-banking seuvices because of Security threat E-banking friends

like Internet theft spamming etc. we still very much widespread. 2. Customers are having a threat of loss of private information due to technical faults. 3. The suck of revealing the financial information of the customers to others. 4. Communi cation thorough an intermet might not be the best base for bank and customer relation as belief hight partially be lost. Augueness among customers about the E-banking / facilities and procedures is stru at lower side in Indian Scenavio. Less amarieness of new technologies and their benefits in among one of the most stanked breveiers in the development of e-banking.

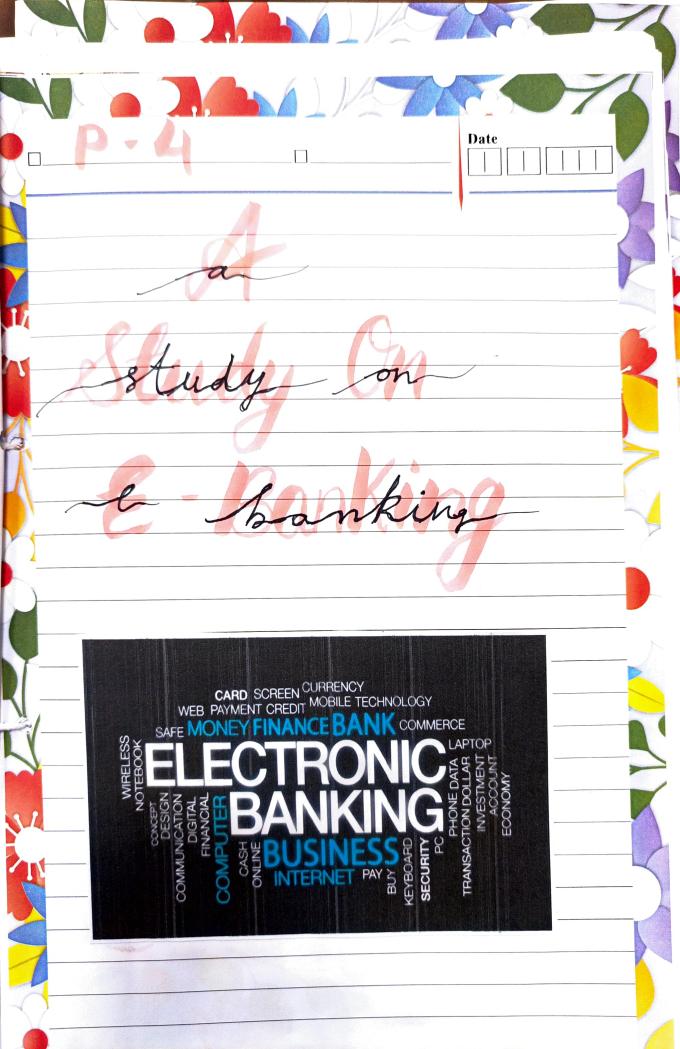
Field Project Working Notebook

S.No. क्रमांक	Date (दिनांक)	Tir fron (समय रं	i–to	Details of the work done (किये गए कार्य की जानकारी)	Signature of the student (खात्र के हस्ताक्षर)	Sign of related Authorities (संबंधित अधिकारियों के हस्ताक्षर)
1	1/2/22	llam	3pm	Introduction of E-banking,	refatel.	
2	2/2/22	llam	Зрт	Types of E-banking	nfotel	
3	3/2/22	1)am	3fm	Types of E-banking.	mate)	
4	4/2/22	llam	3pm	Functioning of Automated Teller Machine (ATM)	Mato.	
5	\$12/22	llam	3pm.	features of Tele banking	mfate)	
6	8/2/20	leam	3pm.	Knowing about the types of cards.	mfatel	
7	9/2/22	Bom	2pm	Importance f function of various cards.	mfatel	
8	10/2/21	10am	2ρm.		mlatel	



9	11/2/22	llam	Зрт	Introduction of the various services.	matel		
10	12/2/22	llam	3pm	knowing about the types of accounts.	Marel		
11	14/2/22	Ham	3pm	Procedures about togging in to the HDFC portal.	Matel		
12	15/2/22	llam	3 <i>pm</i> ,	Procedure of various accounts.	matel	SEC SEC	BANK
13							<4 .6 UR
14							
15							

Total Hours Worked (कुल कार्य के घंटे): __60 Hous.



Declaration of Student's Original Work

विद्यार्थी की मौलिकता का घोषणा पत्र

I MEGHNA PATEL.	hereby declare that
this Field Project report is based on the original	•
published and unpublished material has been us	
declare that the submitted report has not been su	bmitted for any other degree /
course in the past / present.	
में	
घोषणा करती / करता हूँ कि यह परियोजना रिपोर्ट	मेरे द्वारा किये गए मूल कार्य पर
आधारित है, जिसमे प्रकाशित एवं अप्रकाशित सामग्री	ो का प्रयोग विधिवत स्वीकृति के
उपरान्त किया गया है मैं यह भी घोषणा करती / कर	ता हूँ कि प्रस्तुत रिपोर्ट किसी अन्य
डिग्री / पाठ्यक्रम हेतु पूर्व / वर्तमान में प्रस्तुत नहीं किया	गया है
	OATEL
Name of student/ Students (ন্তার / ন্তারা কা নাम)	: MEGHNA PATEL.
Class (कक्षा)	· B. com (Hors) 14 Year.
Roll Number (अनुक्रमांक)	: 121 0052
Signature with date (हस्ताक्षर दिनांक सहित)	: mfatel

Approval letter by guide / Supervisor (निर्देशक का अनुमोदन पत्र)

I hereby certify that the above mentioned report is the actual report of the student's project work	
done under my supervision. It has been submitted to St. Aloysius College (Autonomous), Jabalpur after my approval.	
मैंएतद द्वारा	
प्रमाणित करती / करता हूँ कि उपरोक्त वर्णित रिपोर्ट विद्यार्थी द्वारा मेरे निर्देशन में किये	
गए परियोजना कार्य रिपोर्ट की वास्तविक रिपोर्ट है यह संत अलॉयासियस	
महाविद्यालय (स्वशासी), जबलपुर में मेरे अनुमोदन के पश्चात प्रस्तुत की गयी है	
Van de	
Signature	0-01
Name Dr. Komaj	Kairal
Date 6/4/22 Asst. Pr	nofessa
Date 6/4/22 Place Jabolane Signature Name Dr. Kornal Asst. Pr Commen	Cl

Work Completion Certificate

(After the completion of the work, attach the given certificate on the letter head of the external institution here)



We understand your world

This is to Certify that (name) Meghna Patel Class B: (om. Hons.). of St. Aloysius College (Autonomous), Jabalpur, has completed his/ her Field Project work by being present in this institution from 1st Feb. 2022. to 15th Feb. 2022 and has worked/ trained in the field of E-banking. (Name) Megha Patel is extremely hardworking, dedicated and result-oriented. He / she did good / excellent work during his/ her tenure in the organization. We wish him/her a golden future.
With best wishes
Date: .5/4/22 Place: Jabalpus
Signature of the Authorized Person Deepak Tilousi Name and Seal

Acknowledgement

St. Aloysius' College (Autonomous), Jabalpur would like to express my gratitude towards our Principal Rev. Dr. G. Vazhan Arasu for giving me this great opportunity to do this Field Project Work.
I would like to express my gratitude to my guide teacher Dy. Kornal Rawal Department of
The field project opportunity I had with [Name of the Company] HDFC Bank Vijuunagar was a great chance for learning and professional development. I express my deepest gratitude and special thanks to the [name of authorized person] Decrak Tiwar who in spite of being extraordinarily busy with her/his duties, took time to listen to my queries, to guide me on the correct path and allowed me to carry out my field project at their esteemed organization.
I am extremely grateful to all my teachers and friends who helped me in the successful completion of this field project work.
With great pleasure, I would also like to extend my sincere gratitude and thanks to my parents for their support. I am grateful to everyone who supported me or guided me directly or indirectly.
Signature What Name of the student: MEGHNA PATEL

Index

What is project report?

What are the benefits of project-based work?

About the bank industry

Chapter- i

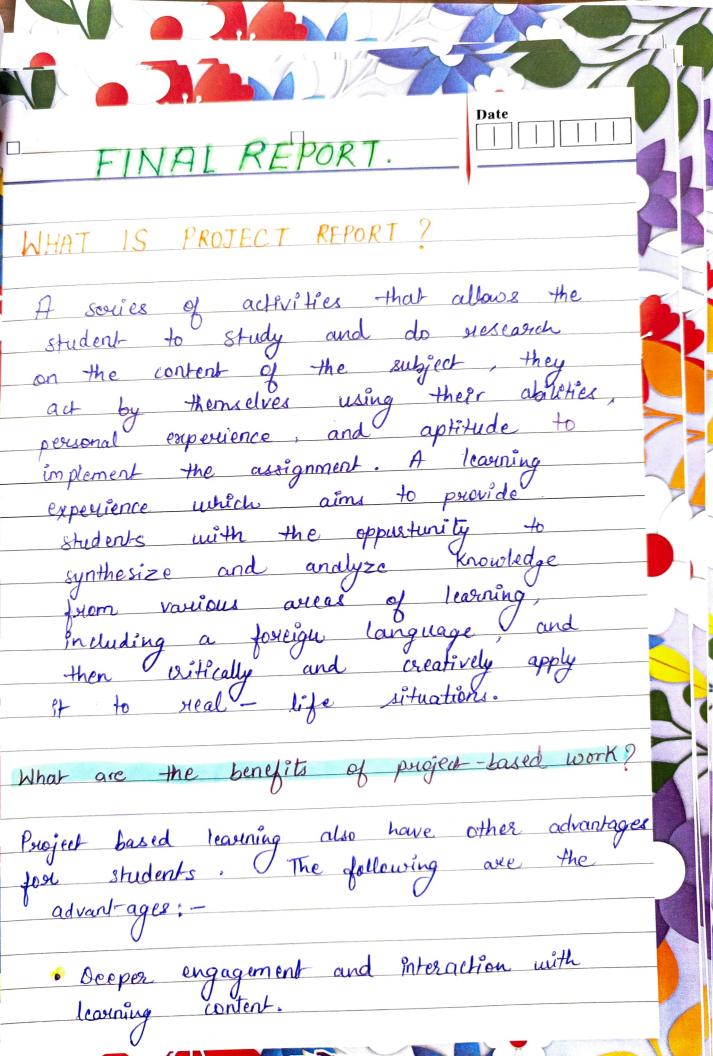
- 1. Introduction and scope of project work
- 2. Background
- 3. Literature review
- 4. Project work plan, relevance and target return
- 5. Details of the concerned workplace/institution

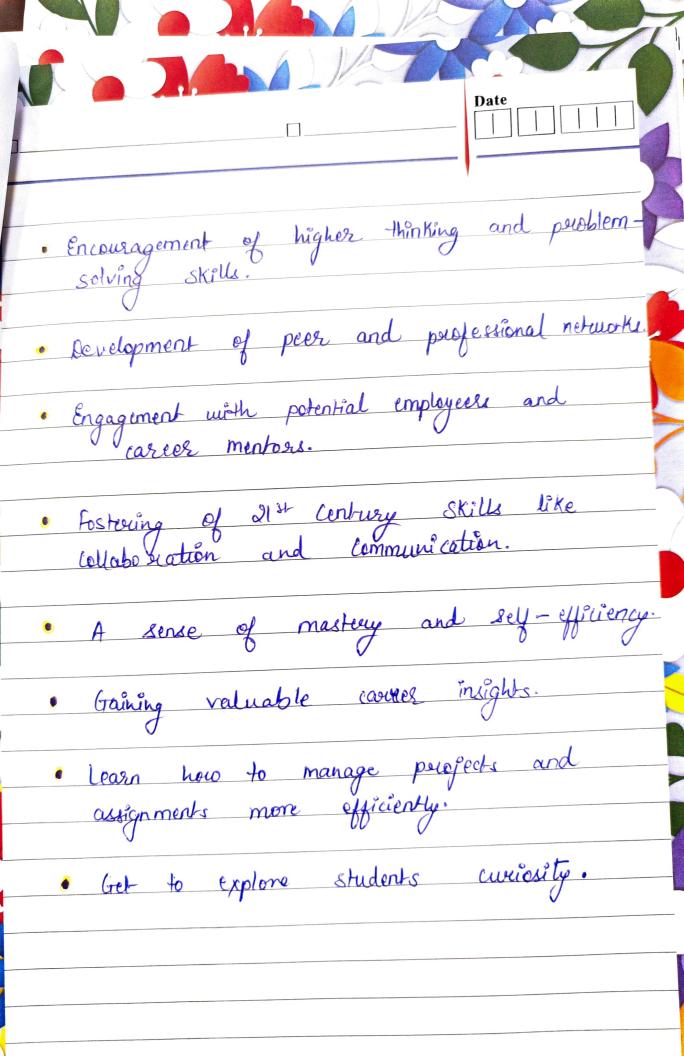
Chapter- II

- 1. Project Work Flow
- 2. Details of Information Collection
- 3. Analysis of Information

Chapter- III

- 1. Conclusions, returns and analysis
- 2. Challenges in project work
- 3. Recommendations

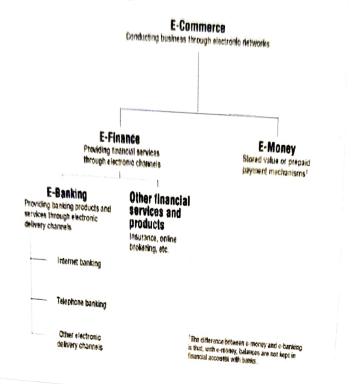


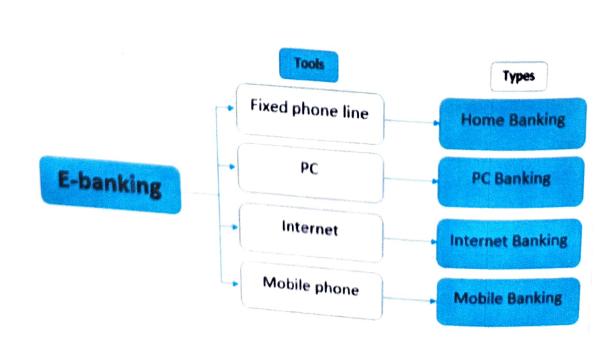


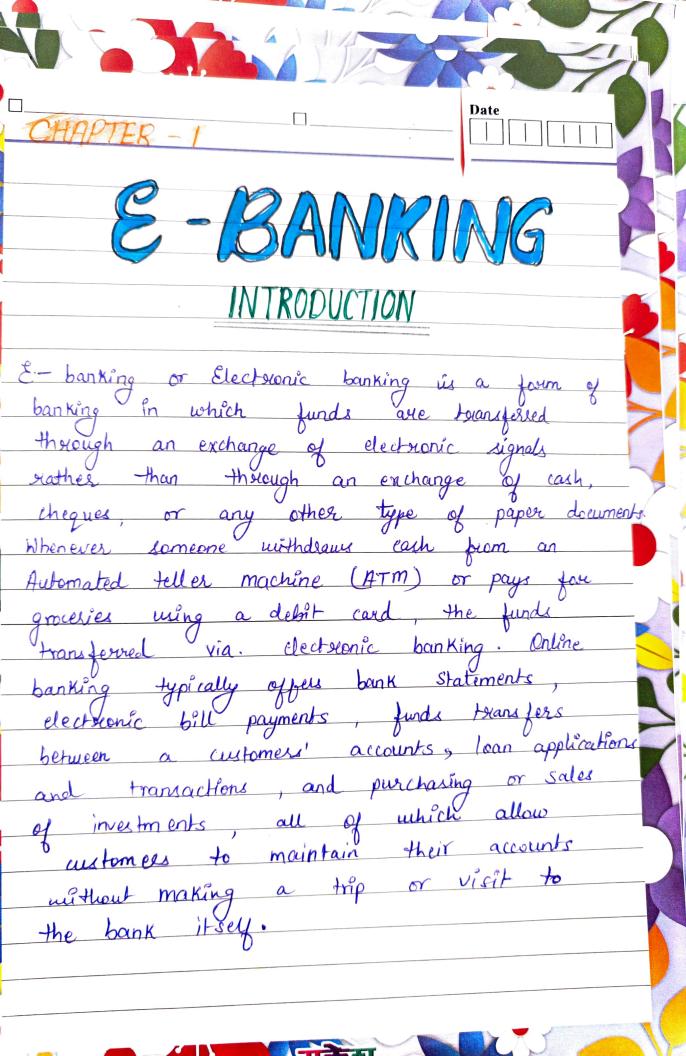


ABOUT THE BANK INDUSTRY Banks play a major stole in the entire Linancial system by mobilizing deposits from howeholds spreads across the nation and making these funds available fox Investments either by lending or buying recurities. Today the banking Inclustry has become an pregues and in critical for the financial wellbeing of individuals, business, nations, and the entire globe. In this study we will see more about the bank. A bank is a financial institution that accepts deposits from public and executes exedit. Principle type of banking in modern industrial world is commercial banking I central banking. Banking can be defined as the business activity of accepting and sufeguarding money owned by others individuals and then lending out to make profit. A banker of a bank is a person, a firm or a company engaged in this business of lending & accepting.

What is electronic banking?







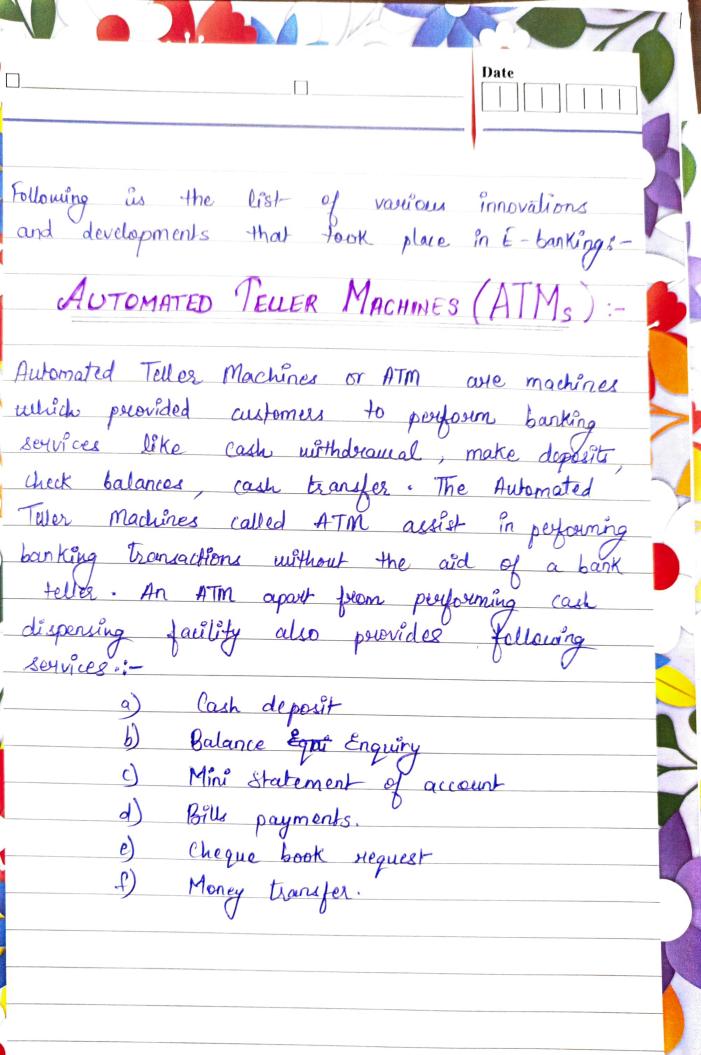
The banking industry has seen many technological changes in the last years which has shaped it from manually intensive industry into one that is technologically dependent. Alternatives to traditional bounch banking have attracted increasing attention as internet usage started to spread over the markety. Bank Started to use the internet for providing new services to their customers. Internet banking System allows individuals to perform banking activities at home via internet. Most of the internet banks are traditional banks which also effer orline banking while others are orline only and have no physical offices. Internet banking transactions states that customers use the internet to got access to their personal or business bank accounts online anytime and to undertake banking townsactions through the use of bank's website . Internet banking allows customers to do their banking transactions anytime and providing control over their accounts.

SCOPE The traditional mode of bounking is manual It is paper based. It is now no longer acceptable in the changing global economy with the underported internet technology, the buiners in also electronic - electronic commerce electronic imposet le enposet, electronic mobile banking etc. The banking sector, if it is to survive in the 21st contury, shall have to change the entine banking stouchuse. It shall have to provide better economical and quick monetary services through the use of advance electronic technology. The customers of the banking are fast becoming computer literate. They are now able to interact more fluently with the prevision of basic electronic services. The needs of the customers have also changed. They require the services of banks at home, in officer, 24 hours a day, seven weeks a day, any where, anytime, and anyhow. The banks one quite alive to the customels.

Date
Date Date
they have fully realized that of they are
camper men shall have
activery of Linguiga Laving L
me are sendering logical
such as electoronic fund townsfer, payments by phone services, personal computer banking
sales transfer, electronic check conversations
etc. The scope of E-banking is
now fast empanding in the developed and
also under - developed countraies of the
world.
& MANAGER STATES
KAKAKAKA KAKA
THE GROWTH OF E-BANKING IN INDIA.
Annual control
Till 1990's banks were adopted to traditional
method ever branch banking. After financial reforms,
the Lanks showed the innovative movement
Januarde hanking services the Indian panning
sectores has accepted computerization since 1993.

India





TELE BANKING:-

Telephone banking is second type of e-banking innovation as this service facilitates the banks customers to perform a sange of financial transactions over the telephone, without visiting any bank branch or automated teller machine. Moreover, the timing of Tele banking is longer as the institution offer 24/7 services to their customers.

These are the following types of transactions which ustomers can perform through their tele-banking-

- i Obtaining account balance.
- ii list of latest transactions
- iii Electronic bill payments.
 - iv. Fund transfer between a customer.

SMART CARD :-

A smart could is also known as a thip could, or an integrated circuit could (ICC) its a pocket - sized plastic could that has embedded in form of computer thip. They are mostly used as payment cards.

FIRM





DEBIT CARD :-Debit could are also known as a bank could. Debit could books like Coredit could or ATM carde, it is a plastic payment card that can be used instead of each when making purchases but operate like cash. Debit cards are different from credit cards as they pay later but debit cards is a may to pay now. pron the customers' account quickly. CREDIT CARD :-A viedit card is a financial instrument issued you (customers') make cashless transactions. The coud issuer determines the credit limit based on your wedit score and your income. Once you get the credit eard bill, you can y supay the amount you have spent within a certain period without any Interest. After this grace period, interestis applied on your balance or repayment amount.





ELECTRONIC BILL PAYMENT

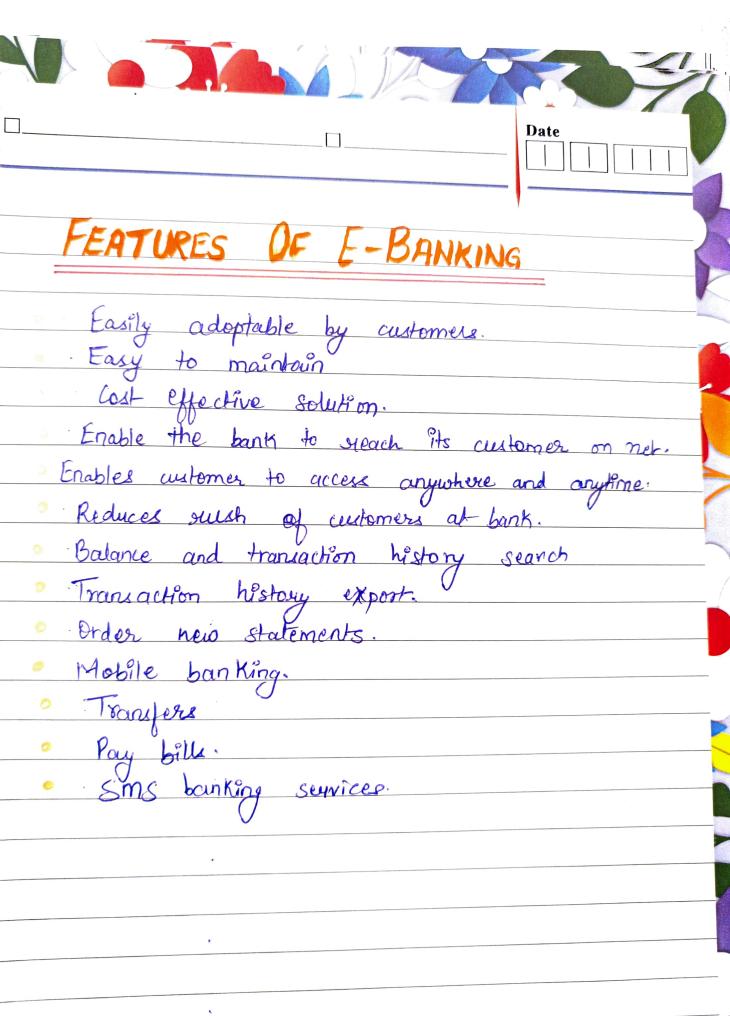
Electronic bill is a type of e-banking innovation that allows a customer to transfer money through financial transactions or credit and account to the creditor or vendor such as a public utility, departmental store or an individual to be credited against a specific account.

Advantages of E-Banking

- · Electronic banking has many advantages making the life easier and banking faster.
- · It saves a lot of time lining up in the bank.
- · Since every bank is connected there are no geographical
- I'mitations letting the operations performed.

 It makes the bank to manage the data.
 - The labour time capital resources needed
- during the transaction.

 Technological developments provide security and privacy to consumer or mytomers.



LITERATURE REVIEW The study reveals that amoveness level of income group above 10,000 per month was high as compared to other income goldings. It was shown that It also depends the size of family. Gender is the courtial issues for the acceptance of internet banking Gupta & Mishra (2012) easuring the new emedging trends of E-banking in the Banking Industry. The study found that there are many chillanges faced by &-banking in banking sector and there are many oppurturities available with the banks. It concluded that the banking sector need to master a new business model by banking building management and customer services. It also suggested that banks should contribute intensave efforts of better service renders to their customers. Chavan (2013) desvuibed the benefits and challanges of Interent banking in an emerging economy. It observed that online banking is now replacing the traditional banking practices. It showed that online banking has a lot of benefits which

add value to customeres satisfaction in terms of botter quality of services offenings and at the same time enables the bank gain advantage ever the competitors. It also discussed some drallarges in an emerging economy. Trivedi & Patel (2014) analysed the problems faced by customers unile using e-banking faulities in India. It observed that the most of the customers know about the e-banking services offered by their bank. The study journel that thous is a Significant diffuence amongst different problems Identified utile using e-banking services: It also found that some problems affect more and some problems after less in use of banking services. It concluded that all the reasons are not equally responsible for not using e-banking services. Hay of Khan (2015) analysed the challanges and oppurtunities in the Indian Banking sector The study showed that only 28 per cent banking clients were using internet banking after the evaluating the population characteristics. It found that there was no significant relationship in between age and use of the upber banking. It also depicted that there is no relation in

Date
between gender and the admis-
between gender and the adoption of internet banking. It observed that qualification in terms of education and income of the
alla Mana
playing the role in the acceptance of online banking.
benking,
but hers biespective on E-1 10
(2011): The enquire rough, sharma Himani
generally use e-banking on services on
presausion of bankery. The bankers are convinced
that e - banking helps in improving the relationship
between the bankers and customers and that it would be a set of
that it would boing patent improvement in the overall performance of banks.
The overall purposimence of banks.

This project work is completed at HDFC Bank within 15 days. The method used to complete the survey in empirical and descriptive in nature. This study is basically based on primary and secondary data, this report Indudes underestanding E-banking in India, introduction, super, background of e-banking also the innovations that I took place in e-banking, and various other details related to e- banking These objective were achieved and realized during the short period of survey at bank. This Study is on C-banking is very essential for customers, through this report we come across the necessity and importance of e-banking in the coming future as it is less time concuming and easy to understand If the person is well educated and is well known with the networks. It is also noticed that the use of e-banking is growing with a great page pace against the customers.



We understand your world

Full Form of HDFC



Housing
Development
Finance
Corporation



DETAILS OF THE WORKPLACE. Shout HDFC Bank This possifect work has been completed at HDFC hank. The Housing Development Finance Composation Limited (HDFC) with the tag line " we understand your world", it is a pointe sever bank. The bank is situated at Vijay nagar, Jabalpur. The whole Survey is completed at this Profitution within the time period of 15 days, It is well known as the premier executive housing Ignance company in India. All branches are Vinked to each other through an online real time bails. HDFC bank provides all types of charking services. These includes: SAVINGS ACCOUNTS: These accounts are primarily meant to inculcate a sense of saving for the future, accumulating finds over a period of time.

Primary and Secondary Products





Advances and Deposits

Loans

Secondary

Cards

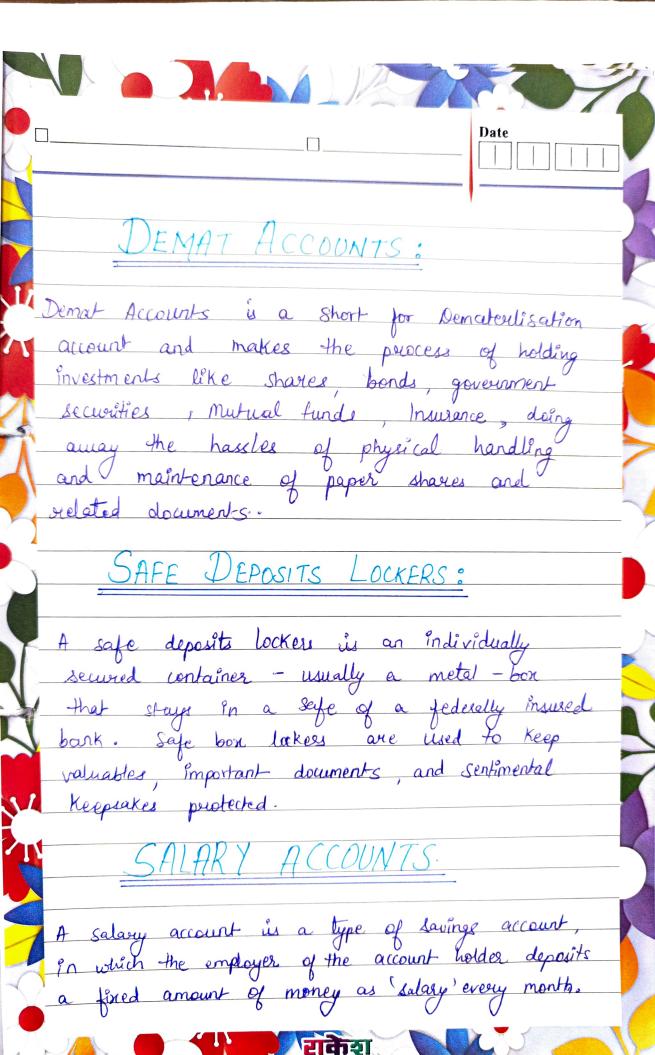
Investment

Forex

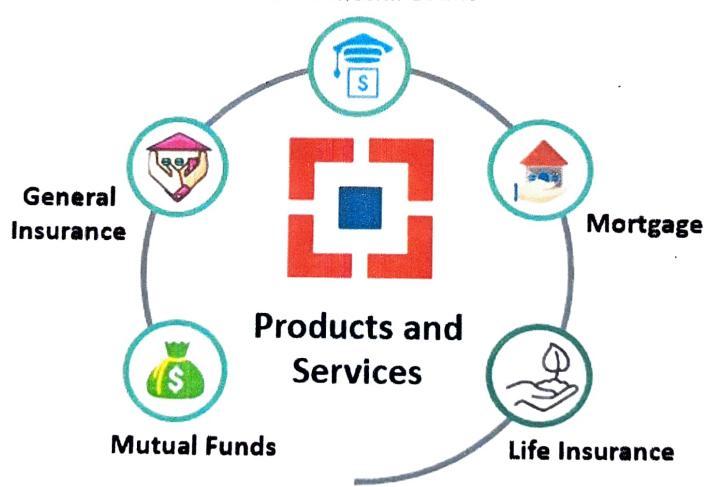
Private banking

Premium Banking

of Savings accounts: -1. Regular 2. Savings Max 3. Institutional Savings 4. Senior Citizens Alc 5. Kid Advantage 6. Corporate Salary CURRENT ACCOUNTS: Current Accounts are very popular among companies tions, public enterprises, buseinessmen who generally have higher number of regular transaction with the bank. FIXED DEPOSITS: It fixed deposit i.e. FD, is an investment Instrument offered by banks to their customers to help save money. with an FD account you can Privest a sizeable amount of money at a predetermined orate of interest for a fined period.

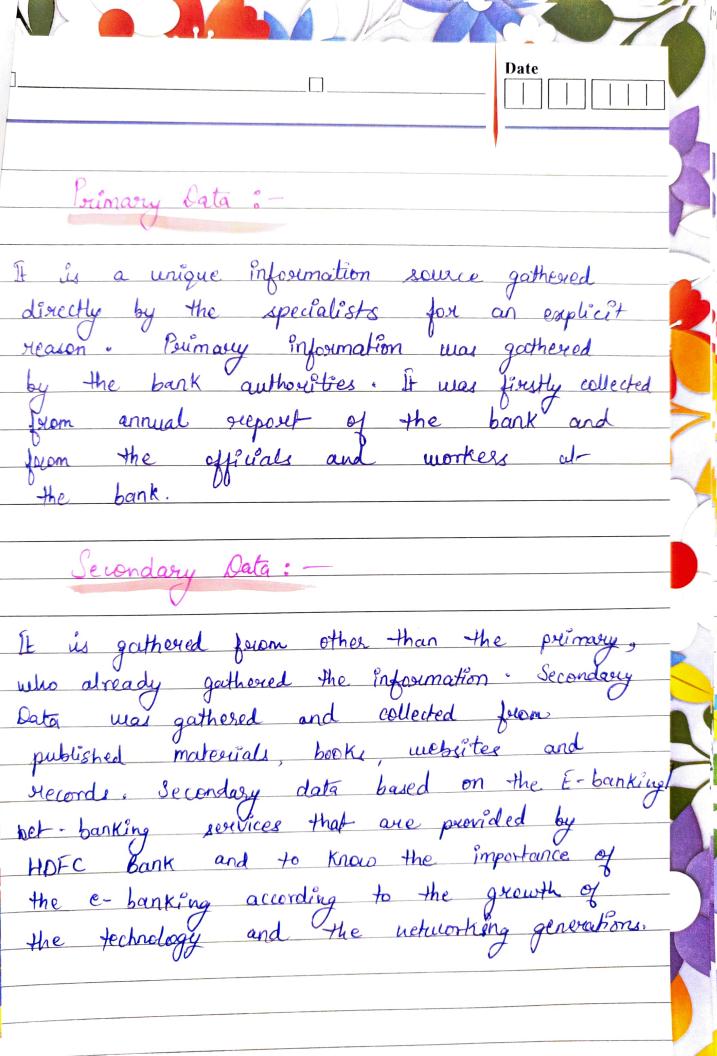


Educational Loans



	2
Date	218
LOAN SERVICES	
PERSONAL LOAN	and the second
HOME LOAN	
VEHICLE'S LOAN.	
GOLD LOAN.	
· EDUCATIONAL LOAN.	
Other services that are cord services (creditioned, debit card), Investments and Insusance	
services, bonds, bills pay, online payment unotesale banking, etc.	

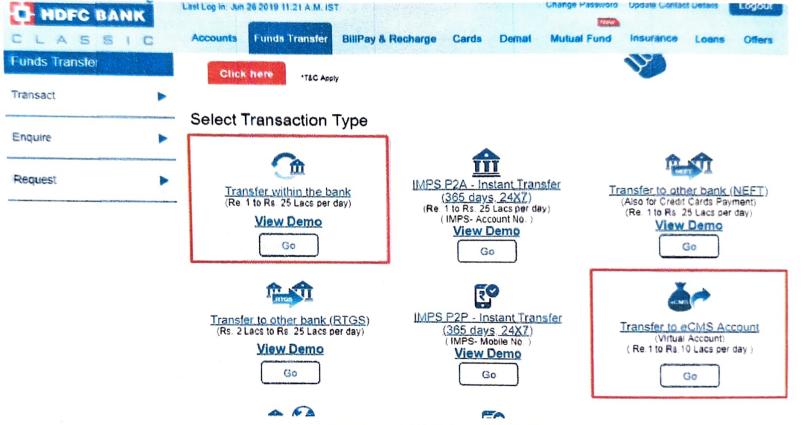
PROJECT WORK FLOW Research methodology is a methodology for collecting all sorts of Information & data pertaining to the data subject in question. The objective is to examine all the issues involved & conduct situational analysis. The methodology include doing field work, collecting information & finally analyzing the information obtained. The mothed both primary 4 secondary data. The porimary data has been collected with the help of bank as well as the personal observation book, magazine; whereas for the secondary data journals and reports has been referred. Data collection is a most essential aspects of any research the whole results depends on the data collected and the information processed. The methodology adopted in this report will be helpful for interpretation of analysis. There following are the types of data collected: -1. Psimary data 2. Secondary data.

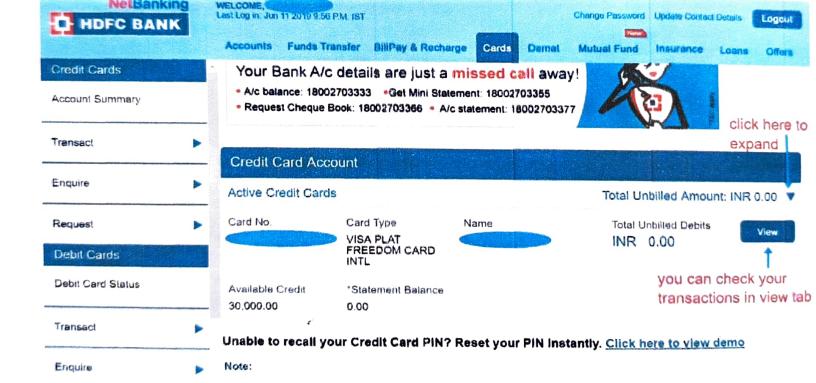


Details of Information collected The following Enformation collected are about the poeducts of HDFC bank and the steps of cestain products: -O HDFC BANK NET BANKING HDFC Bank is one of the largest Private Bank in India to provide Internet Banking Services to retail as well as composiate HOFC bank oustomels. You can avail services like fund transfer, Bill payment, apply for new debit! ceredit coud, open a new FD/RD Account and book tickets and much more without paying additional charges provided you have an internet connection and your mobile number that is neglitered with the book. To avail these services, you must however register for HDFC net banking facilities. · REGISTERATION FOR HAFC NET BANKING. To register, you can visit the bank and fill the HDFC Net Banking Regist-realism Form or else you

you can register through HDFC Bank ATM. Online Registration. If you want to register online through net banking follow the following steps as mentioned below: Phone Banking :-Account holder can also register for for HDFC net bounking seowices by calling HDFC mobile banking numbers. To riegister you need to provide required informations such as customer id 1 Debit and number / ATM number. If the account holder wants to uggister for HDFC net banking through ATM, they need to visit, the neavest HDFC ATM and enter required details such as HDFC detail could number, coud pin setc. Select Net banking registration and follows the steps displayed on the screen.

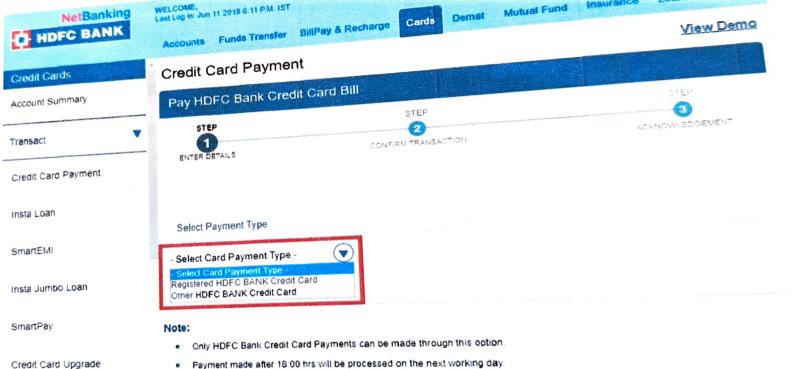
Bank Branch: To register for HDFC net banking facility thoward Bank Branch, download the net banking Hegistocation form from the bank website and submit the duly - filled four with the required documents to the neavest bank branch Accounts - holders can also get the neglist-realton from from the HDFC bank breanch and Submit the same to complete the process of HOFC net bounking. · LOGIN INTO HOFC NET BANKING ONLINE: You can login to HDFC net bounking online, by following the Steps as mentioned below: 1. Visit the official homepage of HDFC bank net banking by clicking on the official website. 2. Now, click on confinue to logen. 3. On the next page, you need to enter your user's name and password and click on login button.





O TRANSFER MONEY VIA HOFC NET BANKING. HOFC customers can transfer funds from their HDFC A/c to another partys account through net benking facility. Before transferving funds years must, add and get the details of the beneficiary account. Following are the steps to transfer funds:-1. firsty you need to login to your HDFC Net banking 2. Click on Fund transfer Tab. 3. Next, select the transfer type as per your regularement, now which on to button. 4. You need to thoose the drawer account benefsvary, Amount & transfer description, etc. 5. Accept the towns and conditions and click on confinue. 6. You'ty the transaction details and confirm. T. Save the reference number for the future référence

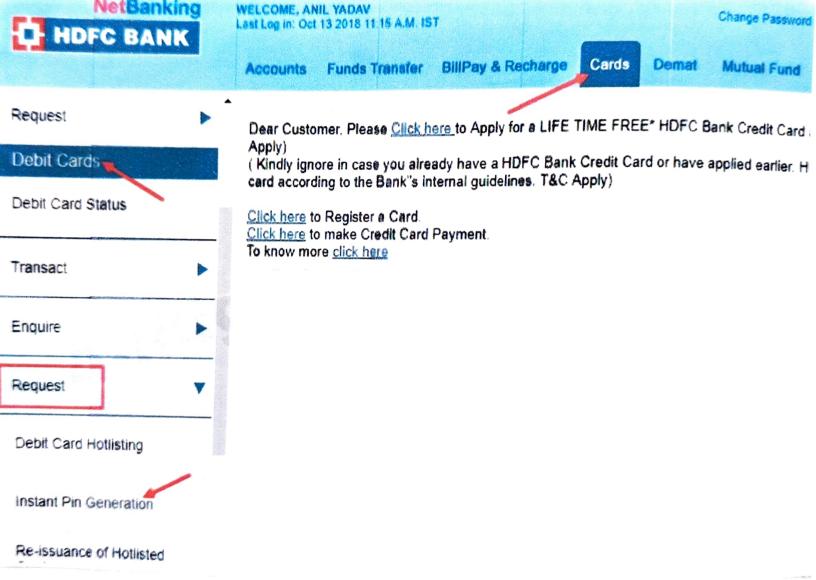
	Date
O CHECK BALANCE VIA HOFC BANK NET	BANKING:
You can also avail Bank Balance checking!	K online
1. To know about HDFC bank balance, you logh to your account on the office of the bank.	
d. Enter your wer name and password.	
3. Now, exter login you can check + bollonie of your account on the banking dashboard.	he bank net
4. You can also know about preevious get a mini - statement etc. by usi feachity.	ng Net Banking



- Payment made after 18:00 hrs will be processed on the next working day.
 - A maximum of 5 beneficiaries can be added

Balance transfer on EMI

Date
O CREDIT CARD BILL PAYMENT THROUGH NET BANKING.
To make oredit rard bill payment through HDFC
Bark Net Barking, you simply need to Register
for HDFC credit could not banking. If you have an
existing sowings account in the bank, the amount will be instantly transferred from the account
when you make the payments.
The following are the steps to make coredit Cord
bill payment throug net banking >-
2 2 2 1 1 10 el 1 1ºeve on the buttle
i) log in to the efficial website by dicking on the portal
2) click on Bill Payment. 13) Complete the registration process by entering your credit
(3) complete the negletration process by entering your credit could detail and clicking on the submit.
4). Enter the OTP sent on your oregistered mobile
Number to complete the negist-reation process.
5). After completing the oregistocation, now to make
payments lick on 'View / Pay Bills' select the
b) Select the Alc where the funds must townsfer , Enter
the Amount to be paid and click on 'Pay Now'.
T) ark on submit to complete the payment.



	Date
O SERVICES OFFERED BY HDFC ONLINE NET	BANKING.
Night Internet banking features, custome request for issues of Demat Dought Books, the opening of new account dose of Loan Accounts.	and cheque
D other services offered by HDFC 1	Bank ove –
• Online Ticket Booking • Opening of FD/RD A/c. • LIC and other Insurance premium • Mutual Funds' Investments. • Credit Card dues payments. • Tax payment income, Securices	
HDFC Benk offers a bunch of products services to meet every needs of the The company cauex for both, individuals corporate and small and medium en For individual, the company has a era accounts, investments, pension schemes types of boars and could that assigned customers. The customers can choose	people. as well as as well a

suitable one from the range of products which will suit their life - stage and needs.

For organizations the company has a host of customized solutions that range from funded services, non-funded services, value addition services, mutual funds etc.

These affectable plans apart from presiding long term value to the employee helps in enhancing goodwill of the company.

During the survey at HDFC bank the information collected wax of E-benking / Net banking and various other services that are available through E-banking. The method used to analyze the data is descriptive and also primary of secondary data is also used to analyze. After the completion of survey and studying the results obtained that are in background of e-banking, importance, features and the innovations that took place, also the services that are

provided through net banking and their procedure and steps to get the results. Internet banking helped improving the profits and reduced the physical presence of the person itself in the banks. It is also considered as the cheapest delivery channel as it is force of cost of charges. It has helped in cutting costs, improving innovations maintaing and improving e-services. Also it is analyzed that mostly customers check the transactions history and their account balances So e-banking has eased the physical presence of customer at the bank to get such simple information about their accounts. E-barking provides faster delivery of sources to Vuide xarge of its customers. Atthough there are also challanges that occurs in e-banking Is that there may be somer problem or the network lacking, so the transactions sometimes get delayed. Even after the challanges the completion of survey gives among the empected sesults: This study conducted is very useful as after the completion of survey gives away the expected nesults and it helped understanding what E-banking is and why is it important

Valle and

and also the services that are available. The yesulte obtained mere upto the expectation and mas ponducted at HDFC bank in a survey.

CONCLUSION The study consists of chapters consisting of Introduction, methodology, review of literature, ovorview of banking industry, details of Net banking, Introduction of HDFC bank, Project Work flow, etc. It gives the new insight to the e-banking users and beneficiaries. Orline barking is just like normal banking, with one big exception you don't have to go to the bank for triansactione. Instead, you can access your account any time and Journ any part of the world, and do so when you have the time, and not when the bank is openbased on the above findings it can be concluded that technology has greatly influenced the bank customers' encouraging them to conduct banking In an innorative manner. They have good austiness regarding ATMs and internet banking. Adoption of ATMs was highest followed by internet banking, credit coved and mobile banking. Variability of of adoption of ATMs and Internet banking is वालेक्या

high among different age groups, here young steris are leading. It is further found that autorieness and adoption of ATMs and internet banking is dependent on education where highly educated have high rates of adoption. Income is closely associated with the adoption of ATMs, internet banking where the high income groups are, ahead of others but the picture is different in mobile banking providing that it is the cheapest e-banking delivery channel.

The research findings clearly suggest that the drive towards ease of banking and convenience is favoured by the customers and therefore banks should find alternative strategic occutes designed to impours service delivery either human based or technology based. It is important to understand that no technology can replace human interface. Technology will facilitate the transaction but it will be the man or woman behind the technology that will matter the most and have the last word.

What Users Expect from a Mobile Banking App



94%

Checking a balance or transactions



58%

Transferring money between accounts



48%

Depositing a check using the phone camera



47%

Paying bills



36%

Finding the nearest ATM or bank branch The study revials about the amoreness and efficiency of HDFC bank net banking practices which will further help bank to review the practices and improving them in father and future, also the findings will bank to chalk more innovative E-banking practices.

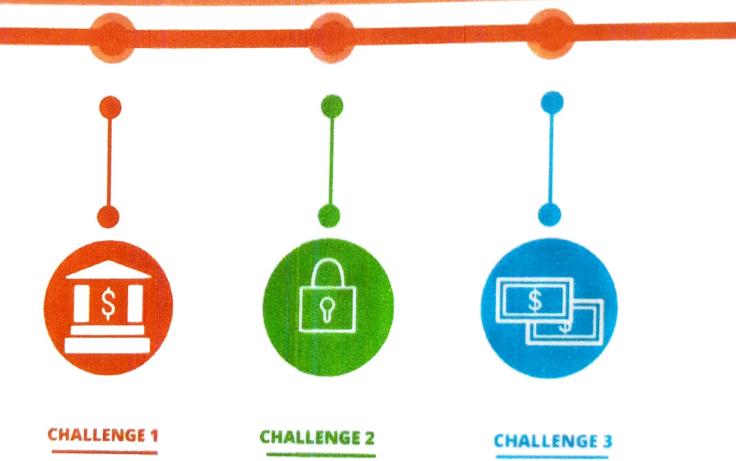
RETURNS/FINDINGS

- In the bankers' view regarding pareference in the net banking usage based on occupation.

 This study revealed that self— employed are at the top and then it is the salaried class who makes the greatest use.
- The study states that most of the despondents are banking with HOFC bank holding the largest percentage of Regulare lavings account.
- The customeres are nearly ready to opt for the net benking in future.

The Study oreveals that large number of customers are not using online banking services because they are not aware and there is no face to- face interactions. The customers who use net banking services mostly perefer both net banking and mobile banking service as there is no charge on transactions It is found that seewer down is the major poloblem in online banking services. Online barking services help to reduce the cost of transactions, save time, they are wer priendly, and they can be accessed from any places. At these features have positive impact on the people for using online banking The process of storting online borking sorvices is very plexible and is easy to understand.

TOP CHALLENGES HINDERING THE GROWTH OF THE ONLINE BANKING SECTOR



TRADITIONAL BANKING HABITS

SECURITY AND FRAUD

INSTANCES

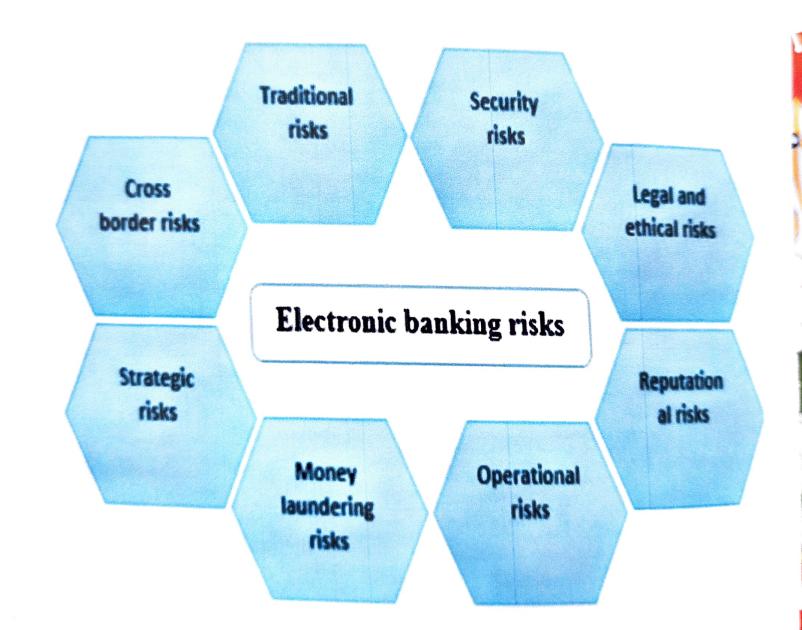
CROSS-BORDER TRANSACTIONS

1 CHALLENGES

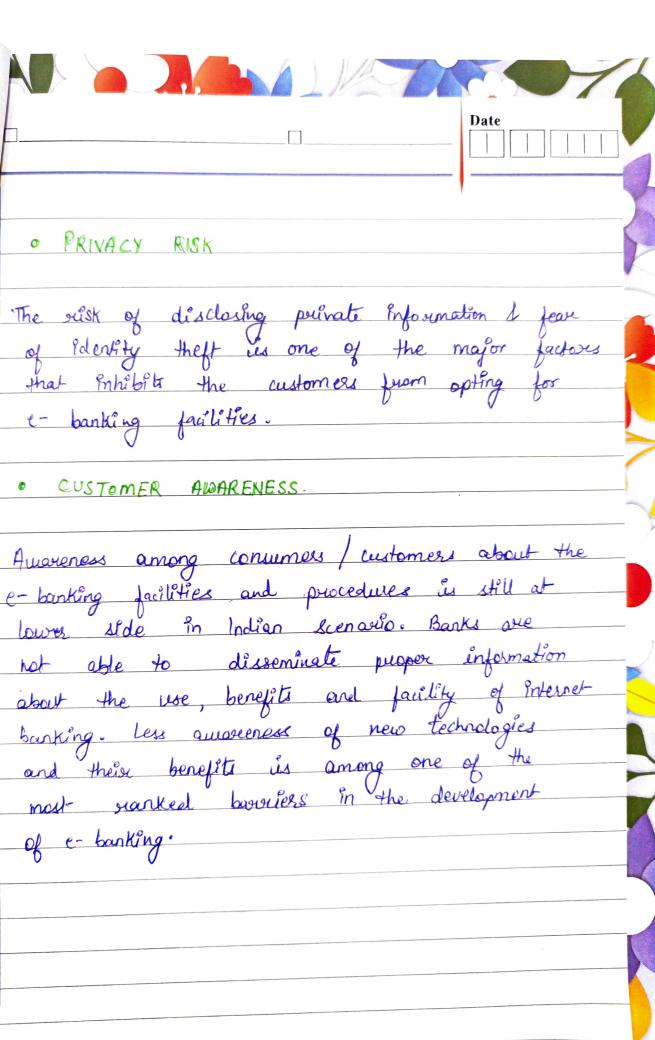
Nowadays electronic banking is a norm rather than an exceptions for the banks. But inspite of it effect numerous assistances for the intomers to make banking easy and convenient but there are many challenges which automets one facing in the adoption of E-banking forme of the challenges are stated belower-

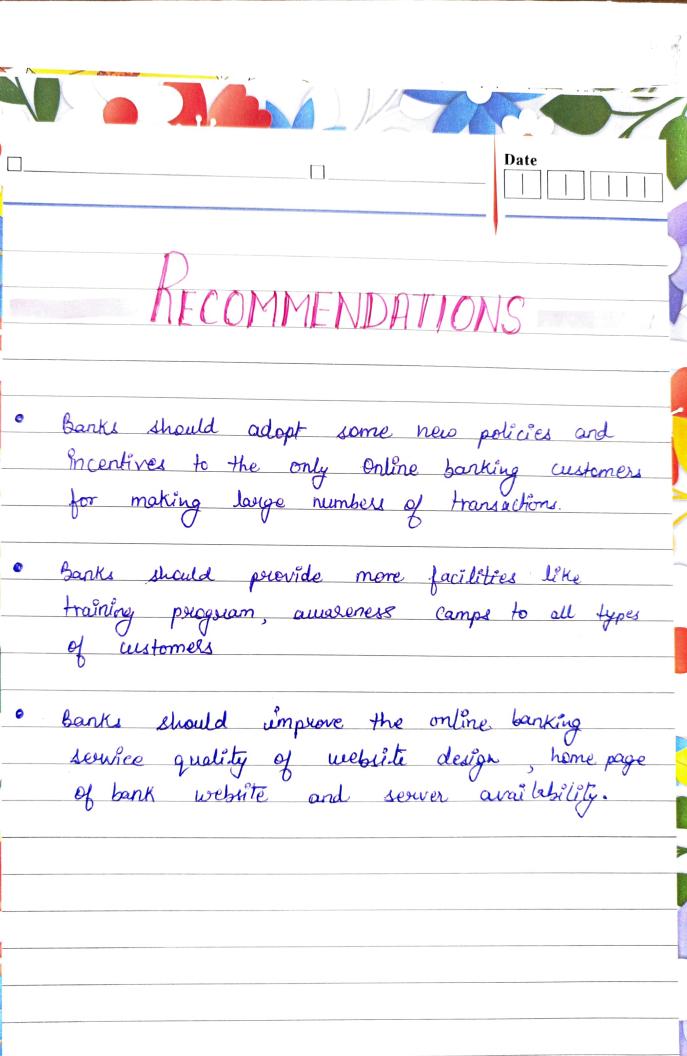
- 1: Customers refuses to adopt E-banking svorvices
 because of security threat E-banking frauds
 like spyrmare, internet theft spamming etc.

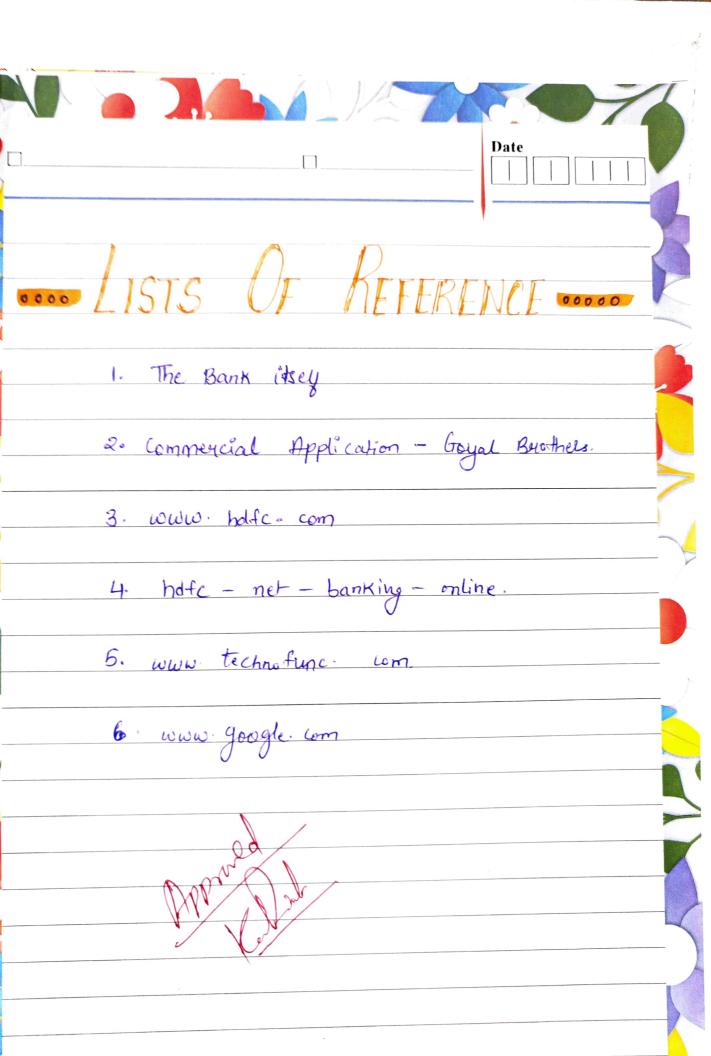
 are still very much underprised.
- 2. Customers over having a threat of loss of private information due to technical faults.
- 3. Insufficient knowledge of using electronic banking and lack of pereposedness by customers and banks in technological adoption.
- 4. Insufficient Infrastoucture for setting up of E-delivery networks.

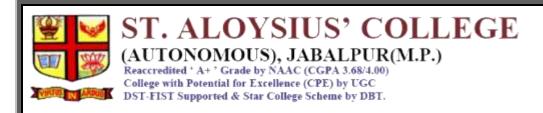


	Date
5. The bankers management, supervisor as authorities are faring several challeng adaption of E-banking.	nd governing es in
6. The visk of revealing the financial of the customer with others, hence are having a fear of privacy is	customels
7. Communication though an internet- be the best base for bank and xelation as belief might prutially be	customers
The most servious threat faced by e-bo	en King is that
may be loss of data due to technical A large number of customers defuses for e-banking due to uncertainity security concerns.	defaults.









SAMPLE PROJECT REPORTS 2021-22 FACULTY OF MANAGEMENT



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REACCREDITED "A+" GRADE BY NAAC (CGPA 3.68/4.00)
COLLEGE WITH POTENTIAL FOR EXCELLENCE (CPE) BY UGC
DST-FIST SUPPORTED & STAR COLLEGE SCHEME BY DBT.

SESSION: 2021-2022

MAJOR PROJECT REPORT ON

A Study of Performance Appraisal Management System in Little Kingdom Sr. Sec. School, Jabalpur

BACHELOR OF BUSINESS ADMINISTRATION

3rd Year

Submitted To:

Dr. Rashmi A. Patras (Faculty Guide) Head

Department of Management

Submitted By:

Rishika Arya B.B.A. Final Year



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Head

Department of Management

Submitted By:

Rishika Arya

B.B.A. Final Year



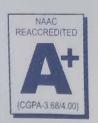
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Email: principal@staloysiuscollege.ac.in, staloysiuscollege1951@gmail.com, Website: www.staloysiuscollege.ac.in



CERTIFICATE

This is to certify that the field study report/project report entitled "A Study of Performance Appraisal Management System in Little Kingdom Sr. Sec. School, Jabalpur" as a Human Resource specialization, submitted by Ms. Rishika Arya in partial fulfillment of Bachelor of Business Administration degree for the academic session 2021-22 has been duly completed as a record of bonafide work under our guidance in practice of St. Aloysius College (Autonomous), Jabalpur, M.P.

This report is up to the standard both in respect of its contents and literacy presentation after being referred to the examiner. With suggestions given, she has done her project by herself.

I extend my best wishes for her future endeavors.

Dr. Rashmi A Patras

Faculty Guide

Head

Department of Management

Date:



Secondary School

DILLIC IVINGUIN DONING

CERTIFICATE

OF APPRECIATION

THIS IS TO CERTIFY THAT MR./MS. RISHIKA ARYA OF BBA 3RD YEAR, ST. ALOYSIUS COLLEGE (AUTONOMOUS) HAS SUCCESSFULLY COMPLETED HER SURVEY IN OUR ORGANIZATION



Mrs. Swapna Rajak

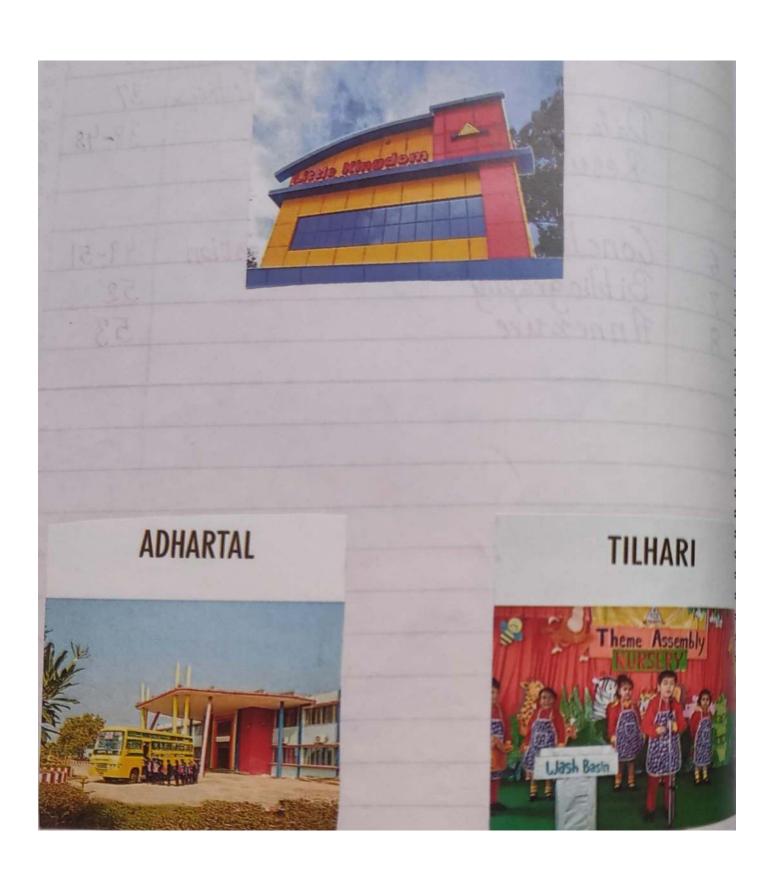
Head of Academics

***** Page No. 1	********
coll No.	Date
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JUCKNOWLEDG.	EMFNIT
J GC K NUW L L D W	L J V L J V I
Rishika Arya, BBA	
Rishira Tirya, DDA	
would like to express	to my Human
hanks of gratitude	to my Human scher Mrs. Rashmi
Resource Vevelopment teo	ant Doorst mont
Resource pevelopment A. Patras, Head of Department Management, for the and support in	ent, Department heir able guidance
Management, gor	completing my
and support in	compring my
project work.	
I would also like	to extend my
gratitude to the He	ad of Academics,
Little Kingdom Sr. Sec.	- School Mrs. Swappa
Rajak, for allowing	me to conduct
Kajak , for allowing my survey in their	
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	Submitted By o
	Rishika Arya
	Rhya

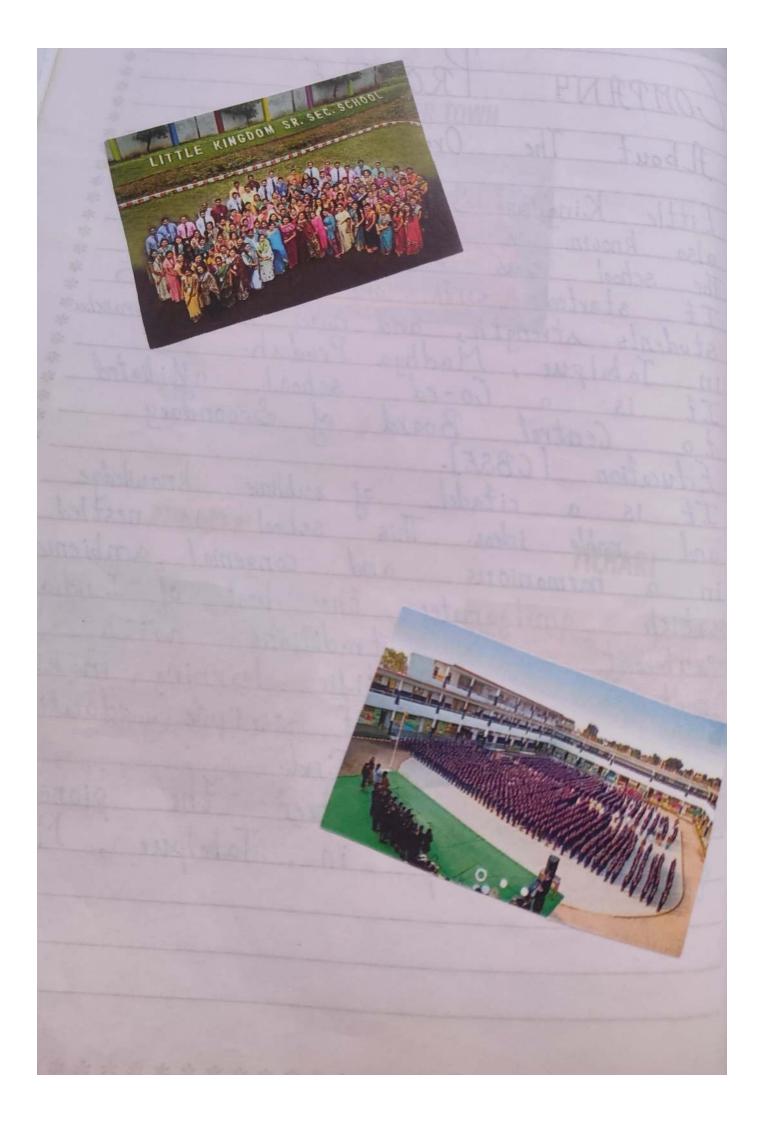
Date
Roll No.
,
DECLARATION:
CLULTINITUM:
I Rishika Arya, BBA final year student;
I historia sincerely declare that
solemnly and sincercity it it is study of:
solemnly and sincerely declare that the project report entitled "A study of the project Appraisal Management System"
Performance Appraisal Management Dystem:
Performance Appraisal Management System: Performance Appraisal Management System: In Little Kingdom Sr. Sec. School" is my: In Little Kingdom Those done this:
own work and I have done this
own work and I have done this project under the supervision of Mrs. Rashmi H. Patras (HOD, Department of
project under the Supplement of:
Mrs. Kashmi H. Patras (HOD, Department
M. M. amon7
T declare that the contine of
1 : DIVEL DOST OF THIS COMPLETED IN
project work and has not been submitted
to any other university for anything.
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Rishika Frya
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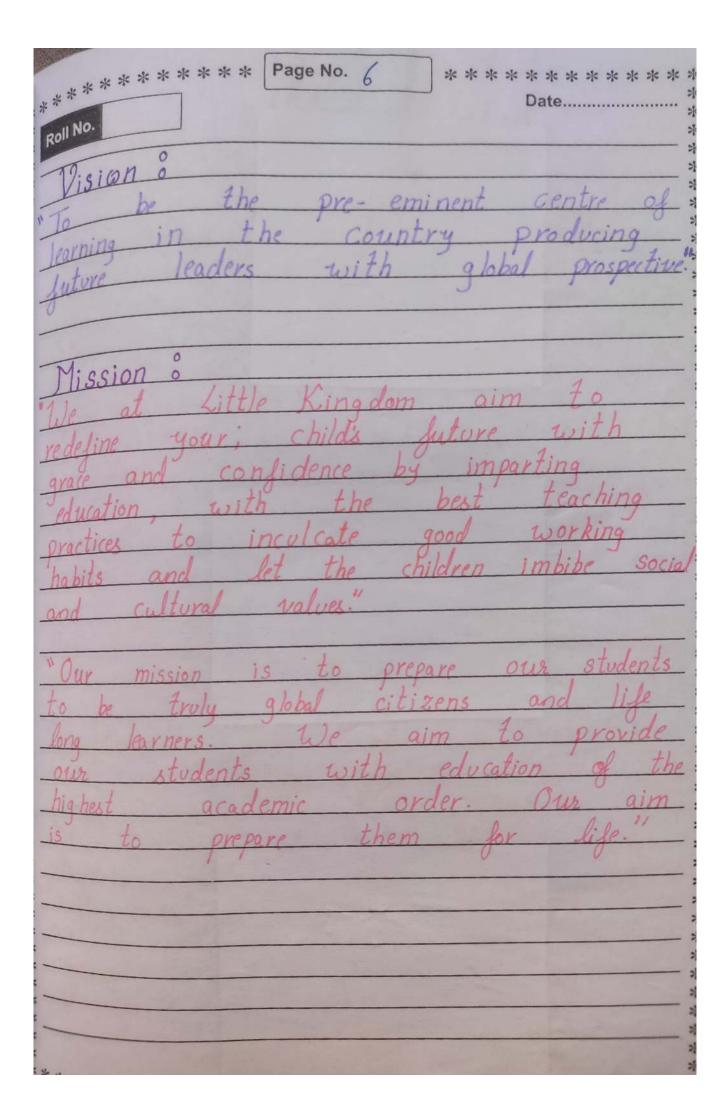
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***** Page No. 4 ********* Date..... * Roll No. * * 35 * * * * 36 * 36 * * 37 * * 38-48 * * * * * 49-5 * * 52 6 * * * nnexure * 8 ******* Chandan *



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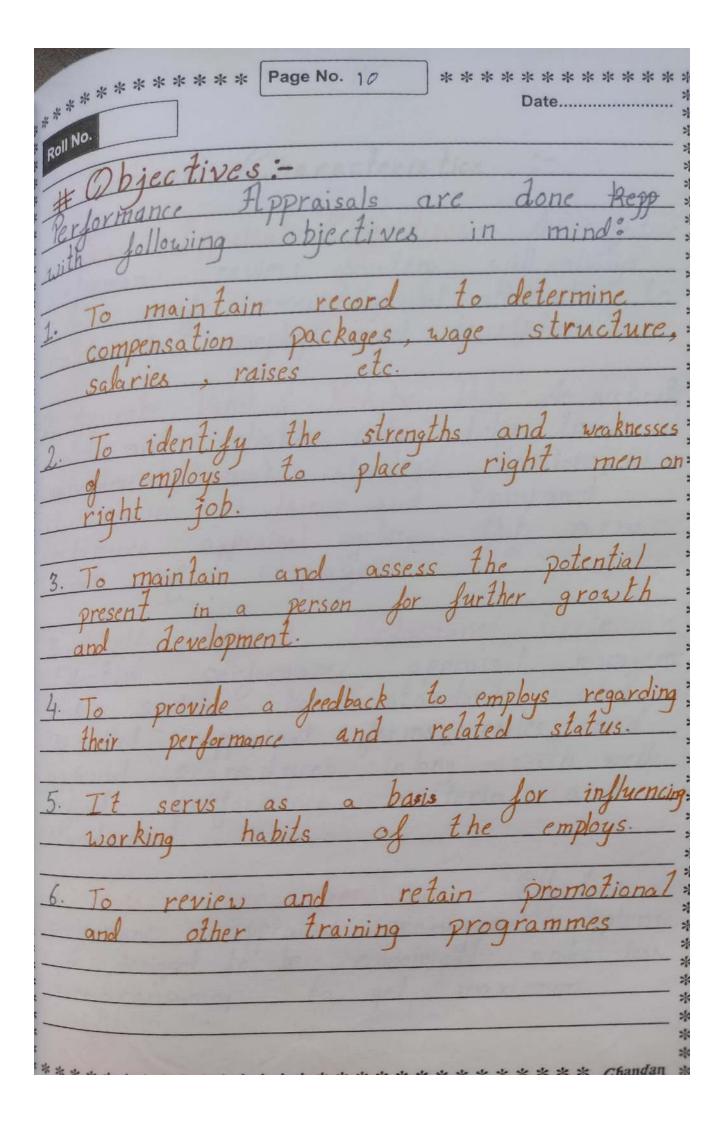




Date.... Roll No. Directors Chair person Schoo Director Gaikwas

********* Date..... ERFORMANGE PPRAISAL

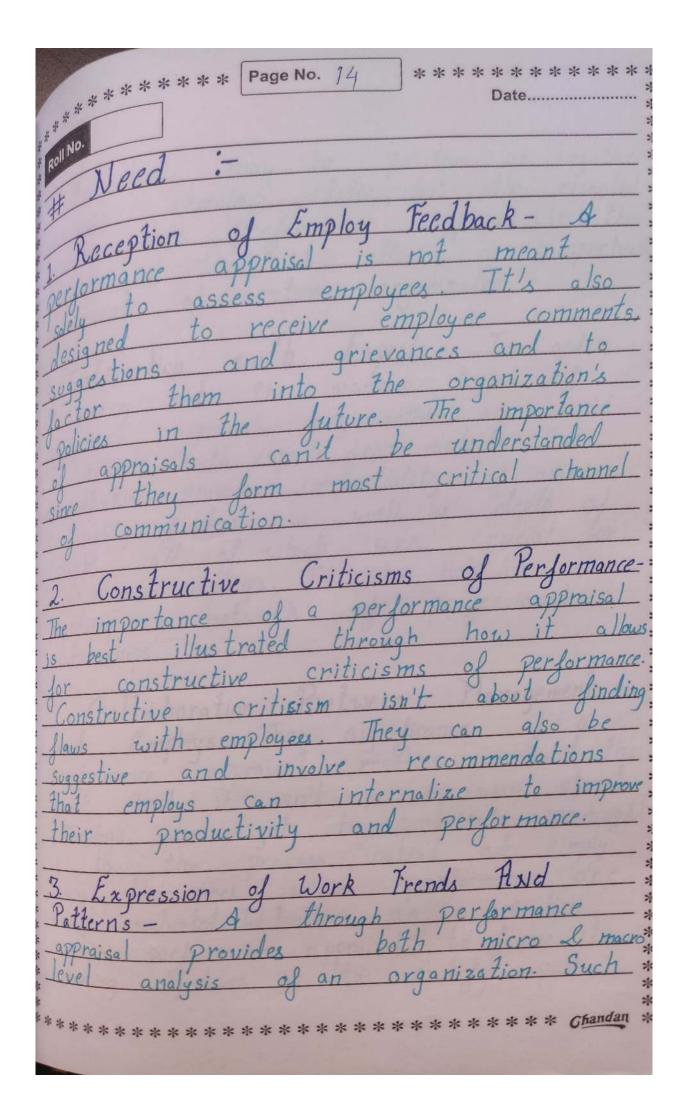
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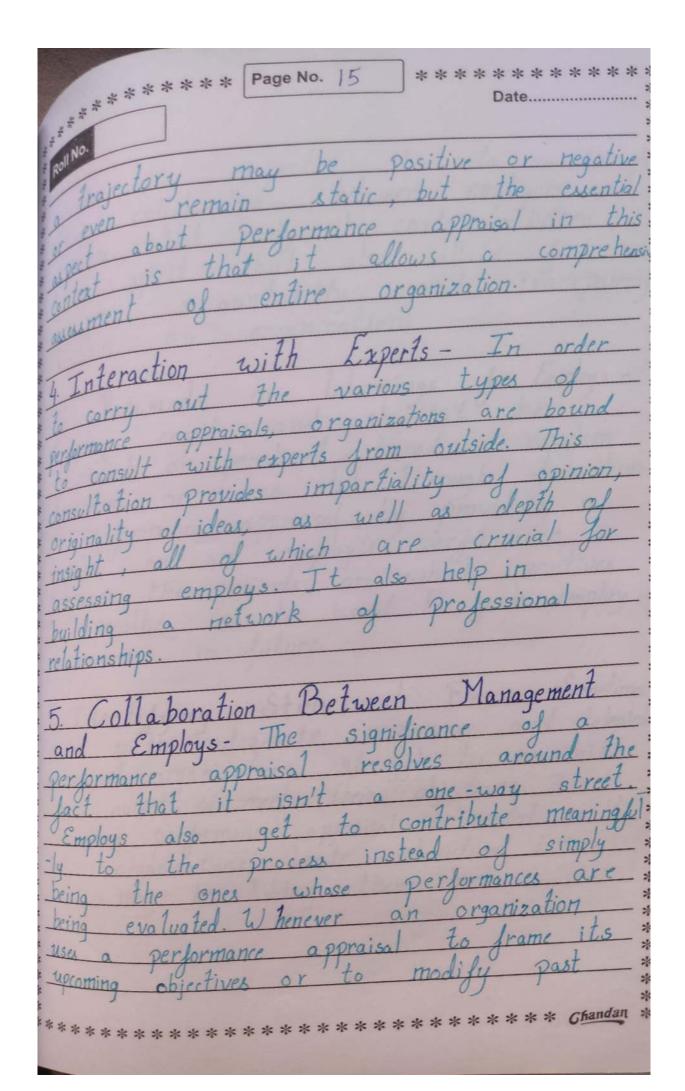


Page No. /\ ***********************************
Roll Varacteristics:
Hopraisal Wojectives - un especiale:
motch the employ's job description.
2. Acurate, Valla & reliable data helps the veliable data helps the veliable strategic decisions, as
oerformance appraisa) system that earns
7 1) ell Delined Performance Criteria-
Effective performance appraisal management system has standards and top-rated appraisal forms, rules, and
appraisal procedures along with well defined performance criteria and standards
4. Less Time Consuming - Effective :
reconsuming to get maximum
benifits.

***** ********* Page No. 12 Date..... SUCCESS ********* Chandan

****** Page No. 13 ********** Date..... ********** Chandan





********** Page No. 16 हैंद और Date..... ewara issued them ********* Chandan

******* Page No. ****** Date..... Vature Derlor mance

*********** Date..... *

Management By Objective (Process Flow)

Plan
1. Define objectives
2. Set control points
3. Choose timeline

Monitor
1. Check Progress
2. Reassess Goals
3. Offer feedback
3. Discuss Out

***** Page No. 21 * * * * * * * * * * * * * * Date..... # Method the ********* Chandan

→ Online objectives of review of re Pre-assessment *Explain the purpose and policies

* Use competency-exercise ma During Assessment Conduct exercise and discussion Note strength and weakness Collect feedback from reviewed

Evaluate the validity of r

Share the feedback with emp

Provide further training it re Post Assessment

******** Page No. 22 ******** Idea easuring senior managemen reason for performance. persormance. finding exercises and Success role. The major For - Manufacturing organizations, => Ideal **** * * * K Chandan

Managerial Self-appraisals 360 - Degree Feedback Customer or Client Review Peer Reviews Subordinates Appraising
Manager [SAM] 12000 日本日本日本日本日本日本日本日本日本日

**** ********* Date..... companies and consulting reason for failure Common method become

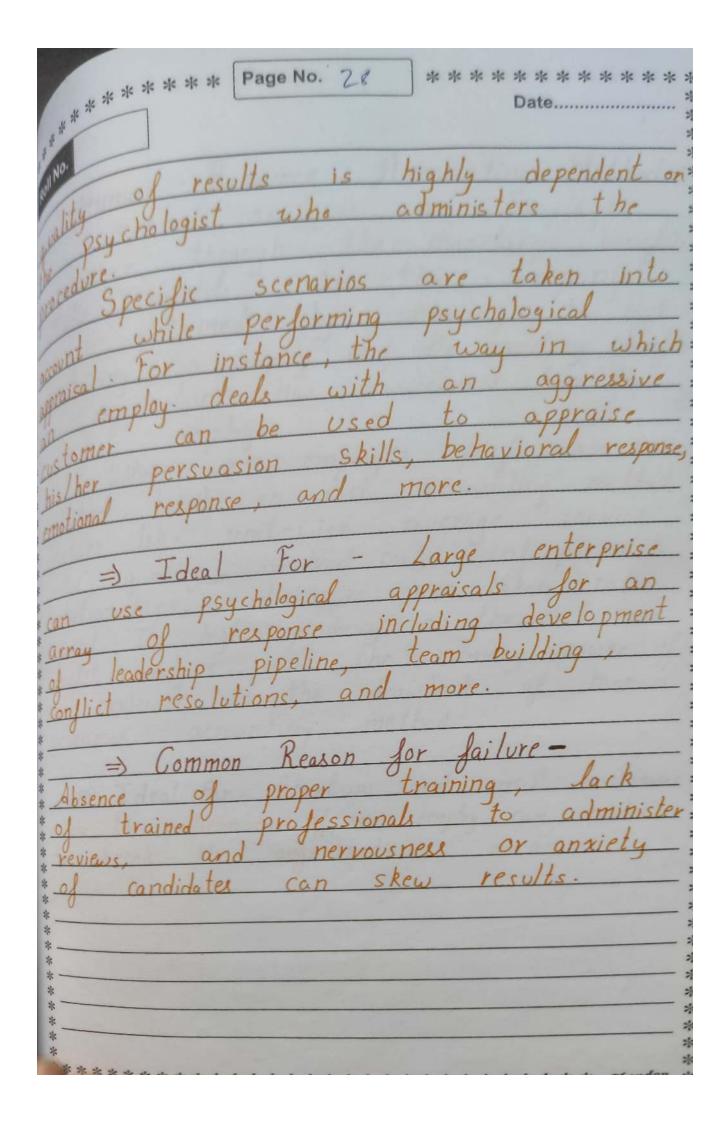
***** Date..... lanagerial Reviews - Performance basic form must by supervisors a team senior managers. Reviews - As hierarchies move organizational picture, coworkers employ's performance ma determine work friendship or animosity between may end up disorting the final Appraising Manager ISAM unique Perspec or

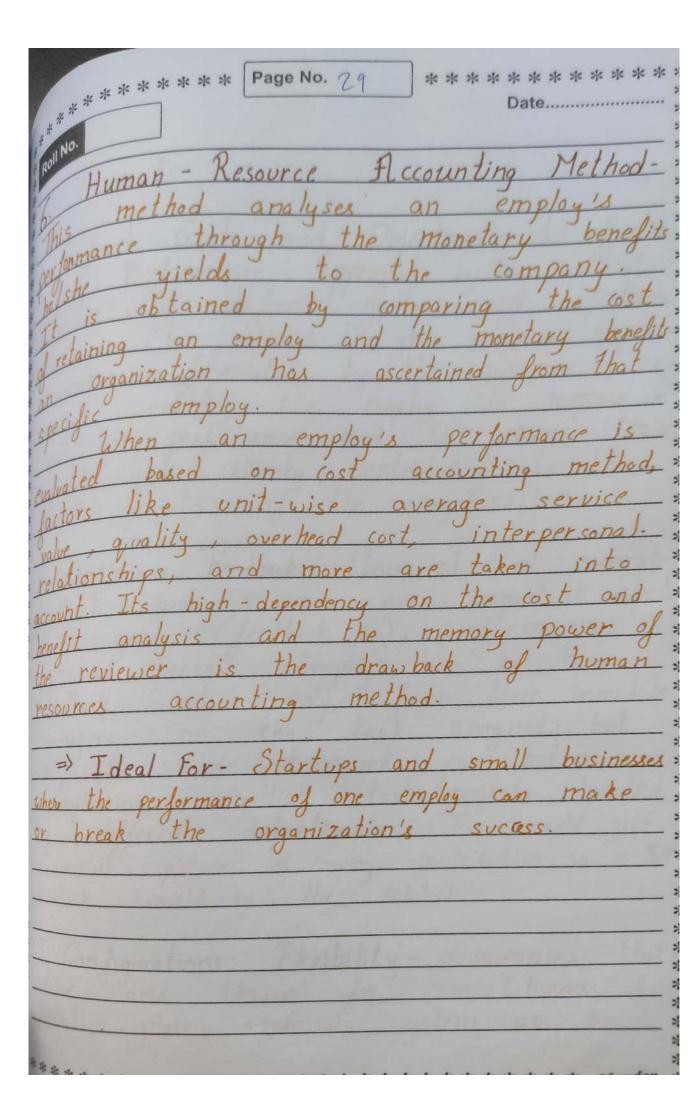
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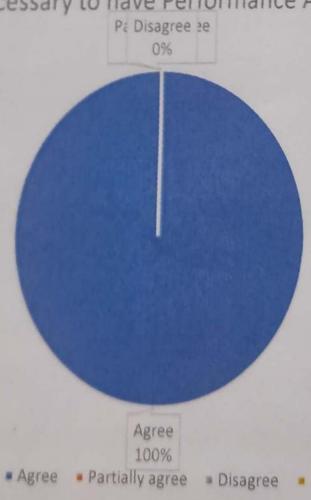
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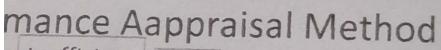
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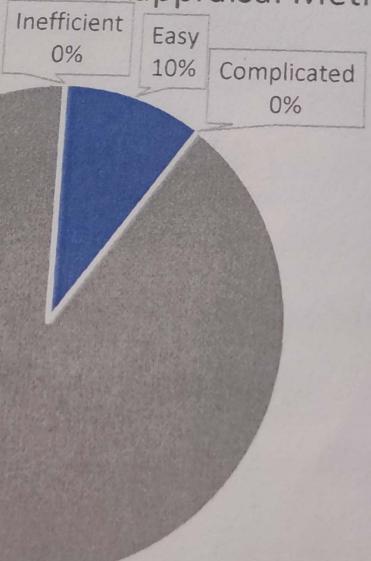
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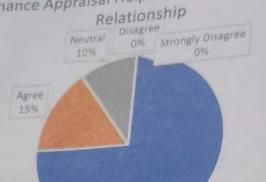
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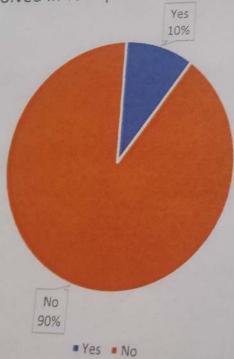
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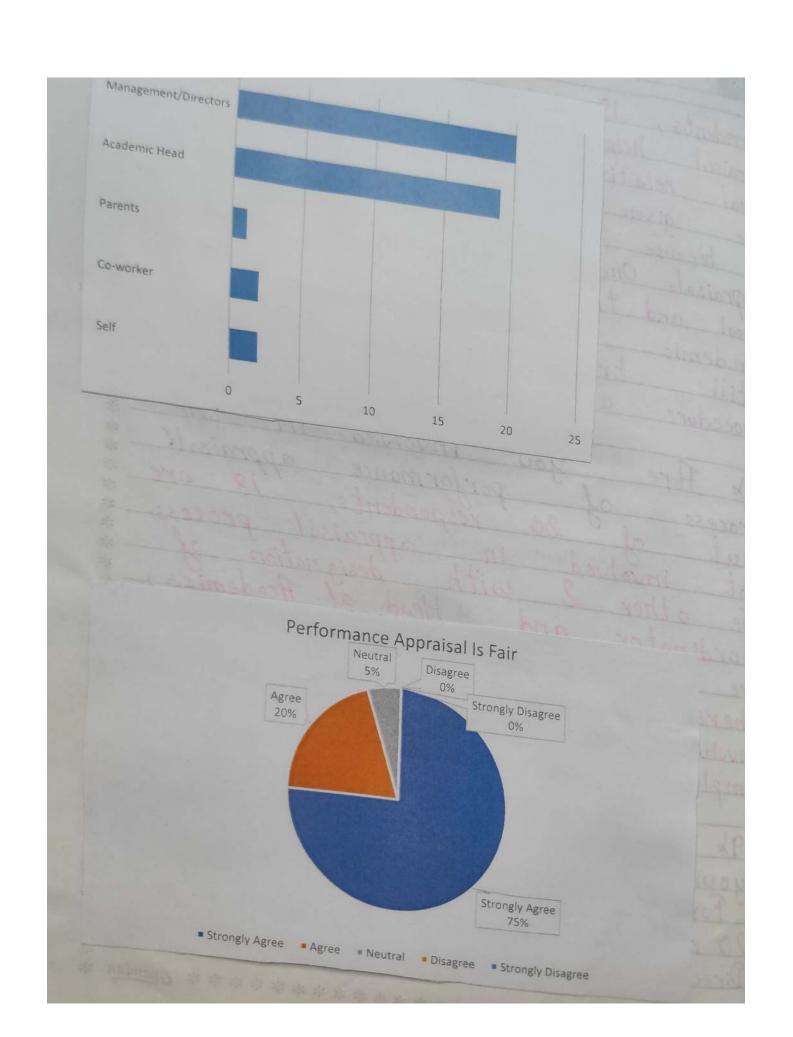
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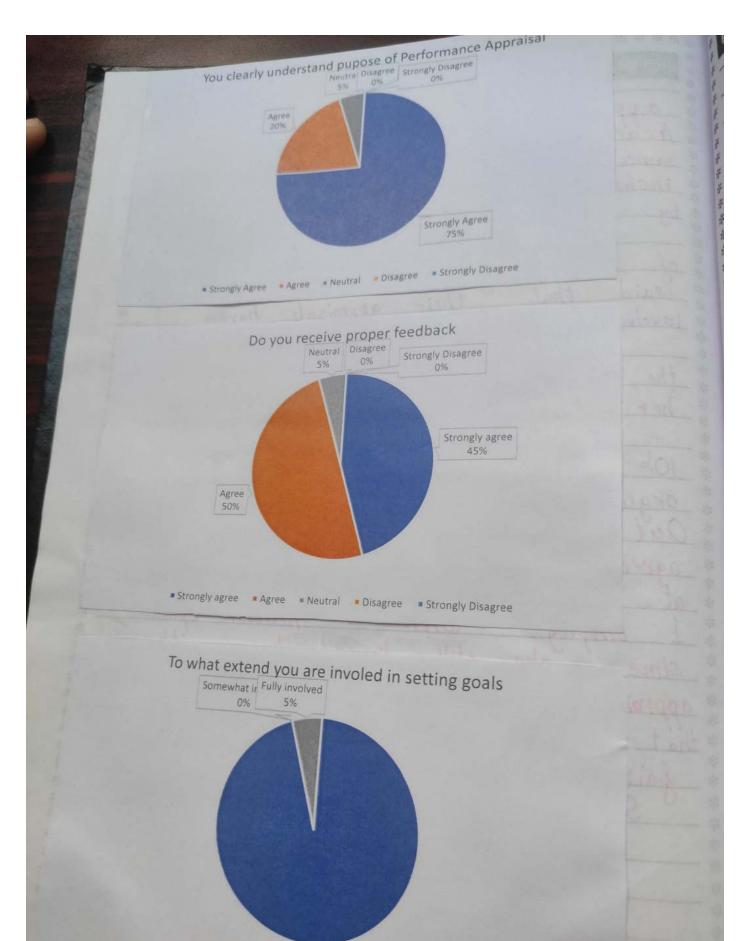
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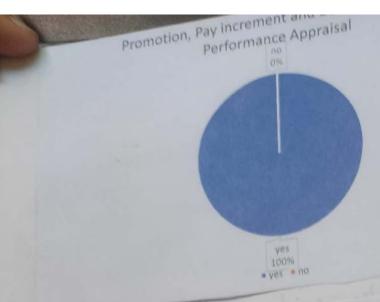


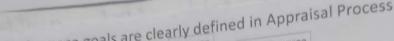
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Not at all in

Page No. 44 ******* Page No. 44 Date
Roll No. I clearly understand the purpose
of performance appraisal. of Again 19 respondents have agreed that they understand the purpose
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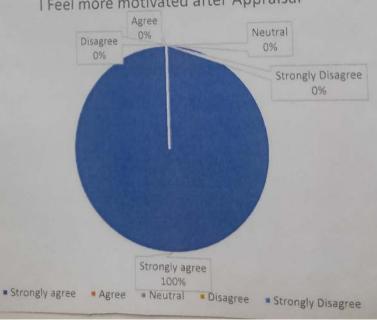
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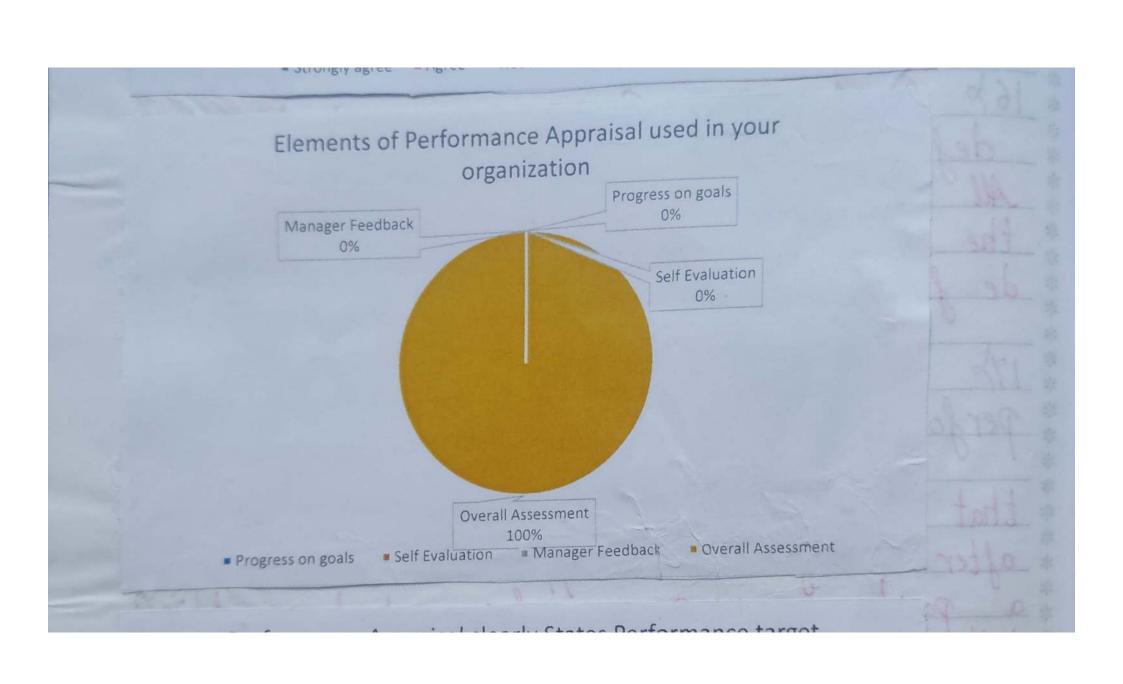
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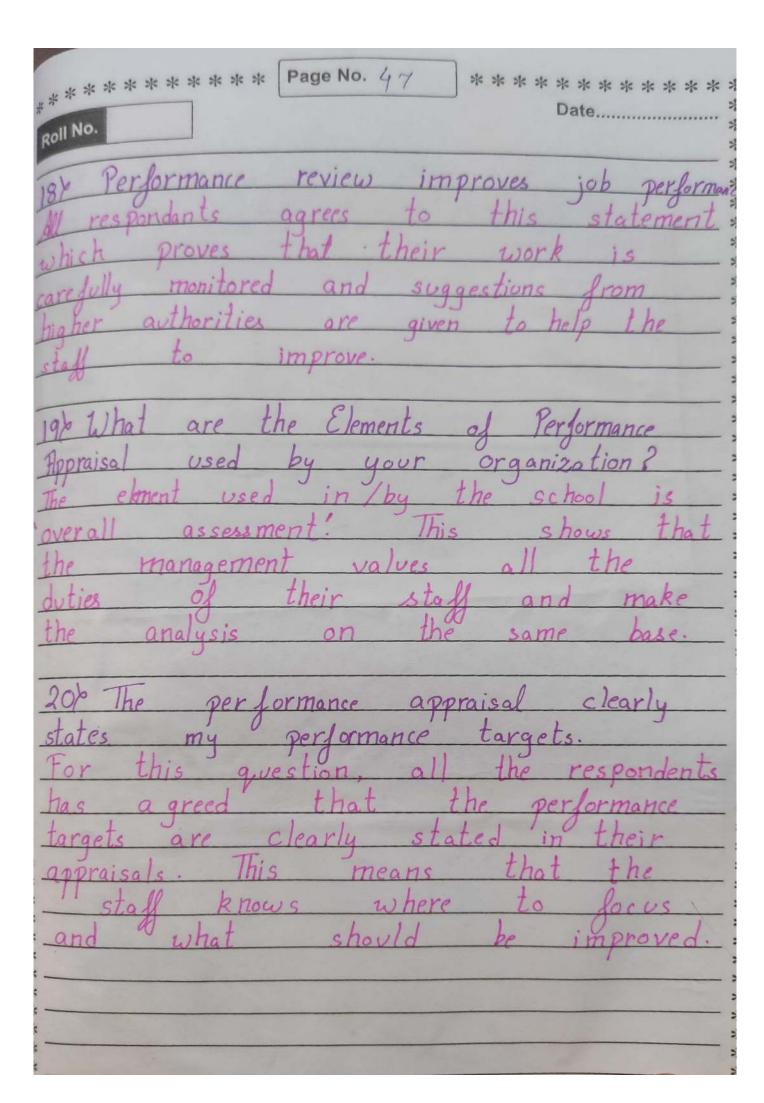


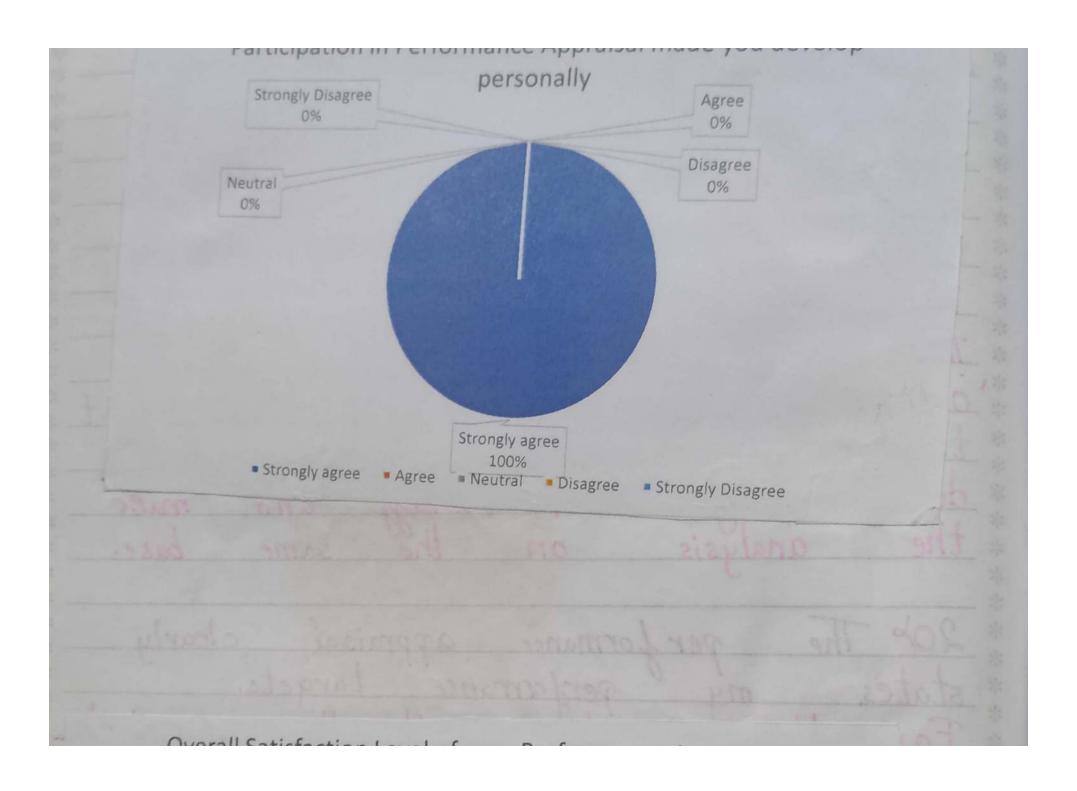
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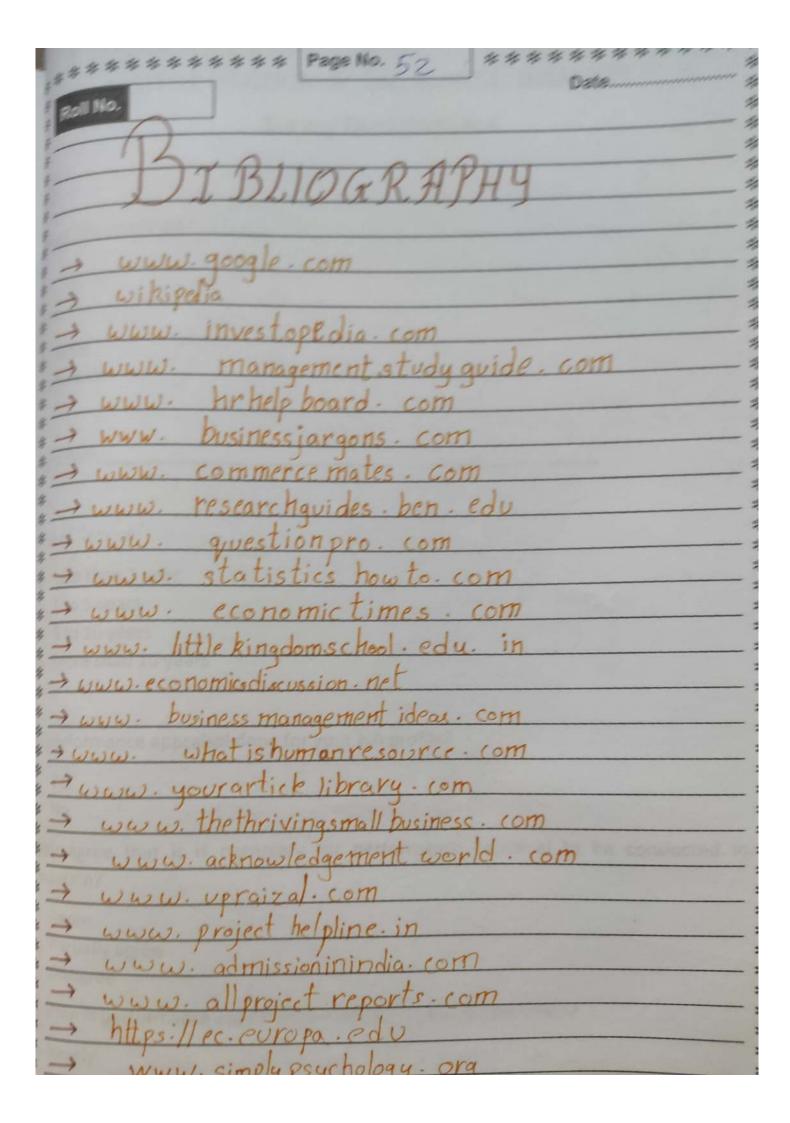
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The employs who have served the organization for less than a year and the employs who joined during pandemic do not have clear idea on appraisal process.
The employs who served the organization for more than 3 years have now became familiar with the process and have now became familiar with the process and have shown a positive view about the same.
Because of pandemic employs didn't got much monetary benifits but enjoyed other advantages.
ower level are involved in their appraisal and in setting goals.
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benefits Appraisal well-define	are al	n, pay I based he	increment on goals	t and Performance are
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UGGESTION Discreption involvement of employs: For the appraisal to reach its potential and be of benifit to all involved there needs to be a higher level of employ involvement in the system than there is at present. One of the key factors of Performance Appraisal was to be have the manager and employ jointly identify ways to improve the employ's performance, and establish a development plan to support the employ in achieving their goals. When employs are involved, they take greater interest as they are given more of a voice. Greater employ participation is known to create an atmosphere of coorperation, reduces tension and rater-ratee conflict which could be caused by the appraisal. 2) Parents and Students' feedback as Criteria of Performance Appraisal: The school should use feedback forms and suggestion boxes to be filled by parents and students about performance

**** Page No. 51 ******** Date..... Roll No. teacher and other measure company forganization-wide: 1SSUE place leve Some more only one years the goals, what and based appraisa The organization should framework of performance appraisal that sets out a number such as fairness, confidentiality and mutual development need to would the process and to ensure managers & employs are quare system works and can stick to guidelines.



A Study of Performance Appraisal Management System in

LITTLE KINGDOM Sr. Sec. SCHOOL, JABALPUR

Survey Questionnaire

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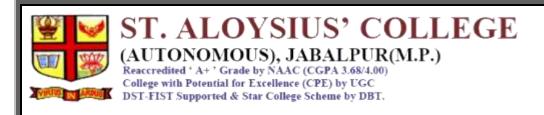
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SAMPLE PROJECT REPORTS 2021-22 FACULTY OF SCIENCE

Page 1

"SURVEY, COLLECTION AND ANTIBACTERIAL SCREENING OF GANODERMA LUCIDIUM"

Dissertation

Submitted for Partial Fulfilment for the Degree of

MASTER OF SCIENCE

IN MICROBIOLOGY

Under the guidance of

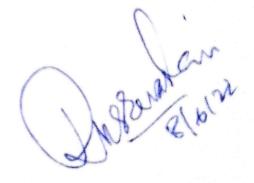
DR. S. S. SANDHU

Director, DIC (RDVV, Jabalpur)

Submitted By

JITENDRA





DEPARTMENT OF BOTANY AND MICROBIOLOGY

St. Aloysius' College (Autonomous) Jabalpur (M.P.)
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SESSION: 2021-2022



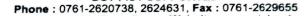
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CERTIFICATE

This is to certify that the Dissertation work entitled "SURVEY, COLLECTION AND ANTIBACTERIAL SCREENING OF GANODERMA LUCIDIUM" is submitted by JITENDRA as a partial fulfillment for the Degree of "Master of Science" in Microbiology, St. Aloysius' College, (Autonomous) Jabalpur (M.P.) for the academic year 2021-2022. The dissertation has been duly completed under the supervision and guidance of Dr. S. S. SANDHU, Director, Bio-Design Innovation Centre, R.D. University, Jabalpur, (M.P.).

Date:

Place: Jabalpur (M.P.)

DR. FR. G. VAZHAN ARASU

(Principal)

St. Aloysius' College, (Autonomous),

Jabalpur (M.P.)





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(Sponsored by MoE, Govt. of India)

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Former Executive Council Member, G. J. University, Hisar.
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Ref. DIC/53

Date 06/06/ 2022

CERTIFICATE

This is to certify that the work entitled "SURVEY, COLLECTION AND ANTIBACTERIAL SCREENINIG OF GANODERMA LUCIDIUM" is a dissertation work carried out by Jitendra, Master of Science (M.Sc. 4th Sem.), Department of Microbiology, St. Aloysius College, Jabalpur has duly completed his work under my guidance and supervision. No part of this dissertation has been submitted elsewhere for any other degree or diploma. He has done his work sincerely.

Prof. S.S.Sandhu

Director (DIC)

R.D.University, Jabalpur (M.P.)

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DECLARATION

AND ANTIBACTERIAL SCREENING OF GANODERMA LUCIDIUM" submitted to St. Aloysius' college, (Autonomous) Jabalpur, M.P. for the partial fulfillment of the degree of Master of Science (Microbiology), is an authentic documentation of my own work carried out under the Supervision of DR. S. S. SANDHU, Director, Bio-design Innovation Centre, R.D. University, Jabalpur, (M.P.). The matter embodied in this dissertation has not been submitted for a degree of any other academic qualification of any University or examining body in India or abroad.

Date: 06/06/2022

Place: Jabalpur

JITENDRA

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I am immensely thankful to 'Almighty' for giving me the courage and making my path easier to develop and intellect to understand Science and its hidden treasure that today I have been able to complete this research work God keep this grace on me forever.

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I am thankful to Hon'ble **Prof. Kapil Dev Mishra**, Vice Chancellor, Rani Durgavati University, Jabalpur (M.P.) for providing me an opportunity to complete research work.

I would like to express my utmost gratitude to my principal **Dr. G. Vazhan Arasu**, St. Aloysius College, Jabalpur, Madhya Pradesh. His dedication and keep strength above all and his overwhelming attitude to help his students had been solely and mainly responsible for completing my work. His timely advice and scientific approach, meticulous scrutiny have enabled me to complete my dissertation work.

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INTRODUCTION

INTRODUCTION

Mushrooms or fly agaric are fleshy, spore-bearing fruiting fungal bodies that are usually produced above ground, or on their food sources. As long as we found records from ancient times, and perhaps long before humans have consumed mushrooms. Our cultural relationship with mushrooms dates back to the time of the tribes where mushrooms were often considered special and even sacred. This deep spiritual connection with mushrooms extends around the world-from Mesoamerica to the deepest parts of Africa and even Europe, people have a long history of using psychedelic mushrooms for ceremonial and religious gatherings.

"Magic mushrooms" have long been used in civilization since the tribal era, but have been largely abandoned in Europe as we transitioned to civilization and culture. However, in the Americas where Aztecs and other Mesoamerican cultures focused on spirituality and religious rituals, magical mushrooms continued to be used until the Spaniards discovered the area. Archaeological evidence suggests that magic mushrooms have been used throughout history. Hallucinogens such as *Psilocybehi spanica* and *Psilocybe mairei* have been found to be depicted in rock paintings and caves in Europe and North Africa. A prominent example is the Tasiri Cave in southern Algeria. Here, 4,444 species of fungi are depicted in a rock painting dating back 7,000 years. The rock painting near Villar del Humo in Spain, also, shows the mushrooms used in the ritual, dating back about 6,000 years (Roy *et al.*,2015).

It was not till 1916 that western scientific technological know-how first took a hobby in magic mushrooms. Dr. William and E.Safford perused Spanish reports, coming across the point out of mushrooms claimed that mushroom of such homes did now no longer exist. By the 1930s, encouraged via way of means of Safford's claims, more than one botanist and ethnobotanists made their manner to Central America to find out the name of the game and to look if Mesoamerican mushrooms had been simply peyote as Safford claimed. In 1955, R. Gordon Wasson have become the primary white guy to take part in a mushroom ritual in Oaxaca, Mexico. Wasson recorded his stories after which later retook mushrooms together along with his spouse and daughter. An year later, Wasson lower back to Mexico with mycologist Roger Heim in which the pair become aware of seven species of hallucinogenic mushrooms earlier than returning to New York. In 1957, Wasson published -Seeking the Magic Mushroom, formally introducing 'magic mushrooms' to the relaxation of the world.

Mushrooms are classified as fungi and have been used in medicinal and food products for centuries. Mushrooms come in edible, medicinal and toxic varieties. Since 1928, after Alexander Fleming discovered *penicillin*, studies on the use of the medicinal properties of mushrooms and the manufacture of drugs from them have been officially investigated and conducted. Today, some mushrooms and their by-products are used to make medicines for a variety of purposes. Lichens were also used in ancient medicine, but in modern medicine fungi and yeast are the main products. Mushrooms may have been used for medicinal purposes in the early days, but after 1928, modern medicine began to extract medicines from mushrooms. *Reishi* or *Ganoderma lucidum* mushrooms are used in ancient Chinese medicine and are considered to be the longest-used mushrooms for medical purposes (Waktola *et al.*, 2018).

In recent years, various kind of mushrooms have been confirmed, and a number of mushrooms have been cultivated for medicinal purposes. G. Lucidium (Polyocracies) mushroom is a medicated mushroom commonly known as Rishi. Ganoderma lucidum is a mushroom traditionally used in Chinese medicine for the prevention and treatment of human disease. Studies on G. lucidum and its products have reported beneficial biological, health-preserving and therapeutic effects. This mushroom is a valuable herb due to its biological activity such as immunomodulation, cardiovascular, respiratory, antitumor, antiviral, antihepatotoxicity. The species showed antibiotic activity against organisms such as Pseudomonas sp. and Bacillus subtilis. Fungal polysaccharide has been shown to possess antioxidant, anti-inflammatory, antibacterial, antifungal and antiviral activities and can be obtained via solid substrate fermentation (SSF) or submerged-liquid fermentation (SLF). However, owing to the inherent nature of the solid substrate in SSF, fungal growth occurs through mycelial colonization of the substrate bed (Nahata et al.,2013).

known as Rishi mushroom. The exterior of the mushroom is shiny and features a dark colour and a woody texture. Other Rishi mushrooms, require their own ideal conditions for their growth and cultivation. G. lucidium belongs to the Rishi family and describes porous basidiomycetes with double-walled basidiomycetes. Its morphological features are the shape and size of the spores, the microstructure of the crust, and the colour. For a long time, it has been used in the Vedic methodology as a preliminary source of medicines for

Classification of Ganoderma inclaims

Reishi is a genus of porous fungi of the family Reishi, and contains about 80 species, and many from the tropics. They are highly genetically diverse and are used in traditional Asian medicine. Reishi has double-walled basidiomycetes, which distinguishes them from other polypore's. They are sometimes called shelf fungi or bracket fungi.

Scientific classification-

Kingdom: Fungi

Division : Basidiomycota
Class : Agaricomycetes
Order : Polyporales

Family : Ganodermataceae

Genus : Ganoderma

P.Karst (1881)



Etymology- The name Reishi is derived from the Greek word's genos "brightness, brilliance", that is, "gloss" and derma "skin".

History-The genus Reishi was established as a karst genus by karst in 1881. It contained only one species, the *Ganoderma lucidum* (Curtis) Karst. Previously, this taxon was characterized as *Boletus lucidus* Curtis (1781) and then *Polyporus lucidus* (Curtis) Fr. (1821) (Carsten1881). P. lucidus species are characterized by having a lacto (shiny or polished) cap and stem, which is Ganoderma. It is a feature that Murrill suspected to be the reason for Karsten's division, as it contained only one species, *Ganoderma lucidium*. Patouillard revised Karsten's Reishi genus to a total of 48 species, 1889 Monograph, including all species with pigmented spores, attachment tubes, and lactate-studded mountains. Brought the classified. Until Marill studied Reishi in North America in 1902, previous studies focused only on European species, including *Ganoderma sp.* for example *Ganoderma lucidum*. Resinaceum Boud (1890) und G. ValesiacumBoud (1895).

Phylogeny- This genus was named by Karsten in 1881. Members of the Reishi family have traditionally been considered difficult to classify due to lack of credible morphological features, excess of synonyms, and widespread misuse of names. Until recently, the genus was divided into two sections-Reishi with a glossy cap surface (like *Ganoderma lucidum*) and Elfving with a blunt cap surface (like *Ganoderma applanatum*).

(https://en.wikipedia.org/wiki/Ganoderma)

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The fungal mushroom has a wide variety of pharmaceutical interest biotic compounds. These biotic compounds can be used against a number of diseases caused by microbes. Mushrooms are the best source of minerals, vitamins and essential amino acids. Therefore, at present a huge work is carried out by research community for isolation of biotic compounds.

In a study, G.lucidium was grown artificially in polypropylene bags using a variety of inexpensive and readily available substrates. Five wood such as Swietenia mahagoni, Dipterocarpus turbinatus, Tectona grandis, Gmelina arborea, Michelia champaca) saws were used as substrates and cultivated with calcium carbonate (CaCO3) and rice or wheat bran, respectively. Wheat bran has been found to be more effective as a dietary supplement than rice bran. However, S. Mahagoni with wheat bran took 6 days, 33 days, 60 days for mycelial growth, primitive formation and harvest, with subsequent yields of 235, 2 g / kg, substrate that showed the highest mushroom yield with biological efficiency is 7.6%. (Roy et al., 2015).

Another research work was carried out to evaluate the inhibitory effect of various Reishi extracts against *Staphylococcus aureus* (MTCC 3160) and *Salmonella Lucidum* cause Typhoid fever by using agar well diffusion method and broth dilution method (MTCC1249). Methanolic extract of mushroom showed higher MIC and Zone of Inhibition (ZOI) (MIC: 50 μg / ml and ZOI: 2). (12 mm) against *Staphylococcus aureus* and typhoid fever Agar well diffusion method. (Dandapat *et al.*,2015).

Suriyatamilan et al., (2022) collected Ganoderma sp. and treated with different solvents extracted in the vacuum evaporator, Soxhalet appliance and analyzed, its bioactive compound and antioxidant and anticancer activity in HepG2 hepatocyte cell line. Methanol extracts of crude Ganoderma lucidum showed inhibitory activity against test organisms such as Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa, and Staphylococcus aureus. Ethanol extract of AgNO3 treated Reishi mushroom showed good inhibitory zone against ENT-infected microbial species but crude extract showed little zone inhibition.

The Ganoderma lucidum was also cultivated in several media using four different concentrations but the best Mycelial growth was observed on potato dextrose medium at pH 5 and 25 ° C, followed by malt growth. Extract, Kirk's medium and molasses and

MATERIALS AND METHODS

3. Material and Methods

3.1. Survey and Collection of Ganoderma Species

In the present investigation, Ganoderma lucidium was collected from different site of Rani Durgavati University, Jabalpur premises in the month of January-February 2022. Healthy and mature fungi were carefully collected for sampling. The samples were brought to the laboratory in sterile polythene bags for further process. The site of collection of Ganoderama lucidium as given in table 3.1.

Table: 3.1 Collection site of Ganoderma lucidium

S.No.	Common name	Scientific name	Collection Site
1			DIC, RDVV, Jabalpur
2	Reishi mushroom	Ganoderma lucidium	Vigyan Bhawan Area, , RDVV,
			Jabalpur

3.2 Purification of Ganoderma lucidium:

After the collection of Ganoderma lucidium from the nature further grow in in vitro condition in the laboratory. For this, the mushroom samples were clean with tap water properly for removal of dust and debris from the outer parts of the sample. The sample were again washed with sterilized distilled water and kept on the Whatman filter paper for the proper removal of water from the sample. After the cleaning of Ganoderma lucidium small parts of it were placed on the PDA plates supplemented with antibiotics. The Petri plates were incubated at 26-27°C for 3-5 days in fungal bacterial incubator. The Petri plates were monitored every day to check the growth of fungal mushroom colonies from the segments. Isolation of pure fungal strains from the master plate were obtained by transferring the hyphal tips to new PDA plate and incubated at 26-27°C.

3.3 Preparation of media

For isolation of Reishi mushroom Potato Dextrose Agar Medium (PDA) was used. For preparation of PDA 200 / gm peeled potatoes were boiled in distilled water for 30 min. After boiling the potato extract was filtered through Muslin cloth, and then make-up a volume of 1000 mL, by adding sterilized distilled water. Thereafter, 20 / gm dextrose and 20/gm Agar-Agar was added in the potato extract and homogenized by gentle heat. Now, the medium was autoclaved at 15 Psi for 20 min or at 121°C. The preparation of media as given in table no. 3,2

RESULT AND MISCUSSION

4. Result and Discussion

4.1 Survey and Collection

In the present investigation, Ganoderma lucidium was collected from different site of Rani Durgavati University, Jabalpur premises in the month of January-February 2022. Healthy and mature fungi were carefully collected for sampling as shown in table no.4.1 and plate no.4.1.

Table: 4.1 Collection site of Ganoderma lucidium

S.No.	Common name	Scientific name		Collectio	n Site	
1			DIC, RD	VV, Jabalpu	ır	
2	Reishi mushroom	Ganoderma lucidium	Vigyan	Bhawan	Area,	RDVV,
	12000		Jabalpur			

Rajput et al. (2016) also collected the different type of fungal mushroom including Reishi mushroom form the different sites of Maharashtra, India and characterized on the basis of their cultural characteristics, colour and morphology of fruiting bodies and spores. Similarly, Alheeti et al., (2020), first record of the isolation, identification and fruiting body production of the Reishi mushroom (*Ganoderma lucidum* (W. Curt.: Fr.) P. Karst) from the Iraqi environment.

4.2 Morphological Identification of Ganoderma lucidium

For morphological identification slide culture technique was used to observe the morphological characteristics of the fungal strain. After 7 days of incubation at 26±1°C, the colonies on growth media were observed under light microscope (Metzer) and on the basis of morphology the mushroom was observed as substipitate to sessile, dimidiate, fibrous to spongy. Pileus flabelliform, surface glabrous, smooth, slightly dented and corrugated, bright to dull, with semiconcentric furrows, more conspicuous to the margin, cuticle light orange, reddish-golden, deep orange, light brown, reddish-brown, dark brown and almost black in some zones, with deep yellow, furrows due to cuticle detaching, covered by a brown basidiospores layer; margin sterile, obtuse, smooth, with groove zones, pale yellow, with yellowish-orange zones, greyish yellow after contact. The fungi were not fully homogenous, with a deep yellow band under the cuticle, light orange to brown toward the tubes, concentrically zonate, with two resinous bands, the upper thickened, both intermittent, - context length, interrupted near the margin. Tubes were long, brown, orange white towards the pores surface, unstratified. Pores 3-5 per mm, 133-171 μm diam, pale yellow, greyish-yellow after contact, angular to rounded, with irregular edge. Stipe was, lateral, smooth, shiny to dull, flattened too cylindrical, solid, fibrous to spongy, concentrically zonate. Basidiospores are ellipsoids to oblong, apex truncate, with apical germ pore, exosporium with sub free to partially anastomosed inter walled pillars, reddish-brown as

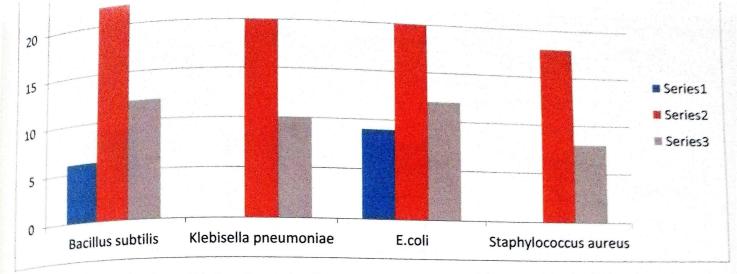


Fig:4.1 Antibacterial activity of Ganoderma lucidium

Vazirian et al., (2014) observed the antimicrobial activity of the secondary metabolites of G. lucidium against Gram-positive bacteria and yeast, with minimum inhibitory concentration values of 6.25 mg/mL. Further the antimicrobial bioactive compound was purified and elucidated as ergosta-7,22-dien-3 β -yl acetate, ergosta-5,7,22-trien-3 β -yl acetate (isopyrocalciferol acetate), ergosta-7,22-dien-3-one, ergosta-7,22-dien-3 β -ol, and ergosta-5,7,22-trien-3 β -ol (ergostrol).

In another research work, antimicrobial activity of various solvent extracts (40Pg/ml) of Ganoderma lucidum was tested against six species of bacteria: Escherichia coli (MTCC-443), Staphylococcus aureus (MTCC-737), Klebsiella pneumoniae (MTCC2405), Bacillus subtilis (MTCC-1789) Salmonella typhi (MTCC-531) and Pseudomonas aeruginosa (MTCC-779). Acetone extract exhibited maximum antibacterial activity (31.60±0.10), while the most susceptible bacterium observed was Klebsiella pneumonia (Sandhu et al.,2010).

4.4 Solvent-Solvent Extraction of Bioactive Compounds

The extraction process is an important step in the investigation of biologically active compounds. When extracting compounds from fungi, the type of solvent used, the extraction process employed and age or type of cultivation of living tissue all can have a marked effect on the type of compound that can be extracted.

In the present study, metabolites of *G.lucidium* was extracted with a number of organic solvent like hexane, methanol, chloroform, butanol, ethyl acetate, carbon tetra chloride, benzene etc. phase (1:1v/v) and concentrated. After evaporation of solvent, the metabolites were mixed with sterilized distilled water and tested their antibacterial activity by Agar well diffusion method. It was observed that fraction of Hexane extract has shown maximum antibacterial activity against *B. subtilis* (20.15±0.70 mm), *E. coli* (18.20±0.80 mm), *S. aureus* (16.80±0.58 mm) and *K. pneumoniae* (16.80±0.58 mm) as given in Table no. 4.4 Fig. 4.2 & plate no. 4.5

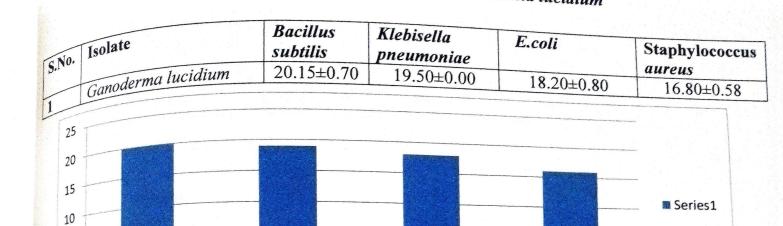


Fig: 4.2 Antibacterial activity of Ganoderma lucidium

E.coli

Staphylococcus aureus

Klebisella pneumoniae

Similar result was found by Desale *et al.* (2013) who observed the antibacterial activity of the crude extract of *G.lucidium* extracted with hexane, ethyl acetate and methanol were tested for their antimicrobial potential. The ethyl acetate displayed significant antibacterial activity against

B. subtilis, K. pneumoniae, E. coli, S. typhimurium, B. cereus and S. aureus.

Chaudhary et al. (2015) also tested antimicrobial activity of methanol extract of secondary metabolite of mushroom strain in a concentration of 100 mg/mL, 200 mg/mL, 300 mg/mL, 400 mg/mL and 500 mg/mL. The methanolic extract was found to show antimicrobial activity even at concentration of 500 mg/mL for *K. pneumoniae* (28±0.00 mm), *S. aureus* (27.33±1.24 mm), *E. coli* (19.33±0.47 mm), *Candida albicans* (10.33±0.57 mm) and *Trichophyton mentagrophyte* (25.66±0.47 mm).

4.5 Thin Layer Chromatography

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Bacillus subtilis

For further purification of antibacterial bioactive compound isolated from the mushroom the thin layer chromatography was done. The solvent-solvent extract that showed the maximum antibacterial activity was run on silica gel G plate in solvent mixture of n-Hexane: ethyl acetate: acetone (4:3:3) that gave a single big spot on silica gel G plate observed after placed in the iodine chamber with a Rf value of 0.60, as shown in Fig.4. The spots were scooped from the silica gel plates and mixed with sterilized distilled water and centrifuged in the micro centrifuge at a rate of 10000 rpm for 10 min for separation of silica gel particles from the compounds. After the centrifugation, the upper layer was separated out into clean sterilized vials and tested for their antibacterial activity. The maximum antibacterial activity was observed against the *B. subtilis* (19.55±0.45 mm), *E. coli* (16.00±0.25 mm), *S. aureus* (16.80±0.58 mm) and *K. pneumoniae* (18.50±0.580 mm) as given in Table no. 4.4 Fig. 4.3

Table: 4.4 Antibacterial activity of TLC spot of Ganoderma lucidium

S.No. Isolate	Bacillus subtilis	Klebisella pneumoniae	E. coli	Staphylococcus aureus
Ganoderma lucidium	19.55±0.45	18.50±0.00	16.00±0.25	16.80±0.58

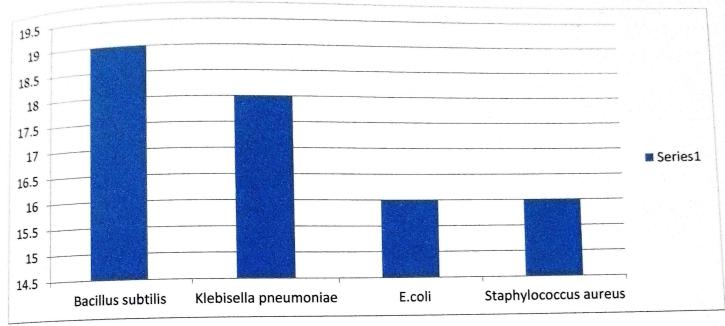


Fig:4.3 Antibacterial activity of TLC spot of Ganoderma lucidium

In another study, Yin et al. (2018) extracted the antibacterial secondary metabolite of the fungal mushroom with ethyl acetate which demonstrated promising antibacterial activity against different Gram-positive bacteria in Disc diffusion assay. The extract was then subjected to thin layer chromatography (TLC) using dichloromethane: methanol (9:1) as a mobile solvent system and eleven spots with diverse polarities were obtained. The TLC chromatogram with bioactive spot was localized with bio autography assay and the result showed that the yellow spot with Rf value of 0.523 exhibited promising antibacterial activity against the four Gram-positive bacteria. In one of the research work an automatic triple development HPTLC fingerprint analysis was carried out for detecting the distribution consistency of the triterpenoic acids in various Lingzhi samples. The chromatographic conditions were optimized as follows: stationary phase, precoated HPTLC silica gel 60 plate; mobile phase, toluene-ethyl acetate-methanol-formic acid (15 + 15 + 1 + 0.1); and triple-development using automatic multiple development equipment. The chromatograms showed good resolution, and the color images provided more specific HPTLC fingerprints than have been previously published. It was observed that the abundance of triterpenoic acids and consistent fingerprint pattern in Red Lingzhi (fruiting body of G. lucidum) ^{outweighs} the other species of Lingzhi (Zhen et al., 2010).

5. SUMMARY

In the present study, Ganoderma sp. were collected from the different site of RDVV, Jabalpur. The morphological identification of the mushroom was done by slide culture technique. After 7 days of incubation at 26±1°C, the colonies on growth media were observed under light microscope (Metzer) and on the basis of morphology the mushroom was observed as substipitate to sessile, dimidiate, fibrous to spongy. Pileus flabelliform, surface glabrous, smooth, slightly dented and corrugated, bright to dull, with semi-concentric furrows. The antibacterial activities of CFCF (Cell-free culture filtrate) of the endophytic fungi were observed for 07, 14 and 21 days by using agar well diffusion method. The fungal mushroom was screened out for antibacterial activity against 4 pathogenic bacteria. During the screening of fungal mushroom, culture free cell filtrates (CFCF) of 7th, 14th and 21st days were examined for their antibacterial activity as depicted in Table 4.2. and plate no.4.3 Based on the result fungal mushroom, gave best zone of inhibition against B. subtilis (22.15±0.70 mm), E. coli (20.50±0.25 mm), S. aureus (18.80±0.58 mm) and K. pneumoniae (20.50±0.58 mm)

In the present study, metabolites of *G.lucidium* was extracted with a number of organic solventike hexane, methanol, chloroform, butanol, ethyl acetate, carbon tetra chloride, benzene etc phase (1:1v/v) and concentrated for the partial purification of antibacterial bioactive compounds. It was observed that fraction of Hexane extract have shown maximum antibacterial activity against *B. subtilis* (20.15±0.70 mm), *E. coli* (18.20±0.80 mm), *S. aureus* (16.80±0.58 mm) and *B. pneumoniae* (16.80±0.58 mm). The solvent-solvent extract that showed the maximum antibacterial activity was run on silica gel G plate in solvent mixture of n-Hexane: ethyl acetate acetone (4:3:3) that gave a single big spot on silica gel G plate observed after placed in the iodine chamber with a Rf value of 0.60, that was further observed for antibacterial activity against the test bacterial strain. After the observation of antibacterial activity of the bioactive compounds and partial purification of the secondary metabolite of *Ganoderma lucidium* which has further sent to the ITC, Chandigarh for chemical characterization of the bioactive compound through Gas-Chromatography and Mass spectroscopy analysis. Hence the bioactive compound of the *G.lucidium* will play an important role in the development of future antibacterial drugs and also has other potential for pharmaceutical industries.

Future Prospects

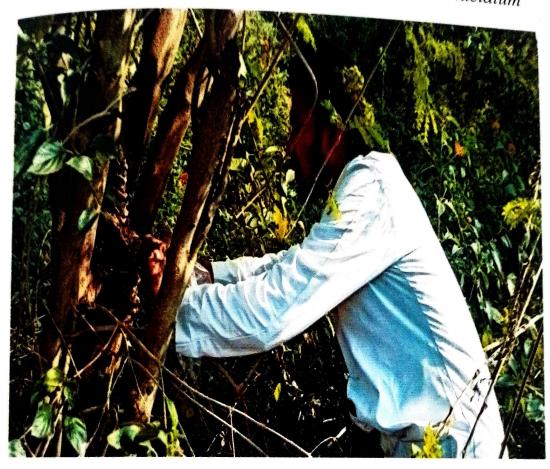
Ganoderma sp., under the extensive in vivo and pharmacological research, can be used in various microorganisms and parasitic diseases. Therefore, future work should be focused on in vivo and pharmacological assays of known compounds, especially Ganoderma lucidum that have antimicrobial and anti-parasitic properties. A better understanding of the antimicrobial and antiparasitic compounds from Ganoderma sp. is crucial for identifying the potential side effects and trace out the new host target and molecular mechanisms, which will provide evidence to further elinical applications of these compounds.

Challenges including genetic analysis, biosynthetic metabolism, separation, isolation and identification may be encountered. In addition, due to the rapid emergence of drug resistance in microorganisms and parasites, fewer options have been left for the treatment of diseases caused by microorganism and parasites. To fight back this problem, further research should be focused on this field for all the identified compounds and the unidentified compounds, which are on the way to be identified.

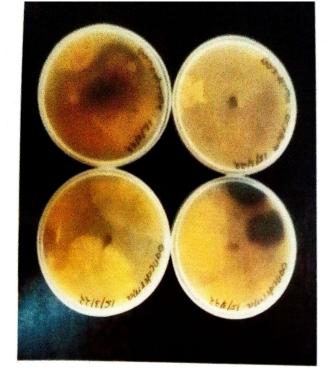
Our work revealed numerous extracts of Ganoderma sp. exhibit the inhibition to microorganisms including parasites, indicating that Ganoderma sp. in particular still seem to possess opportunities for new drug lead compounds. Therefore, further studies need to be carried out in order to explore this concealed area.

No doubt, it is evident that Ganoderma sp. is going to serve as one of the potential sources of novel antibiotics and anti-parasitic drugs in the near future. To reach the apex and specificity of effective antimicrobial and anti-parasite activity, cooperative investigations need to be carried out in the areas of genomic, bioinformatics, chemistry and pharmacology. Moreover, strategies to evoke the sleeping gene clusters linked for the production of bioactive compounds and its regulation need to be adopted.

PLATE: 4.1. Survey and Collection of Ganoderma lucidium







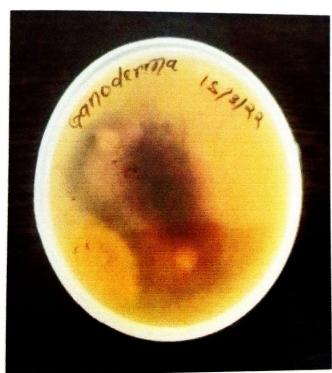
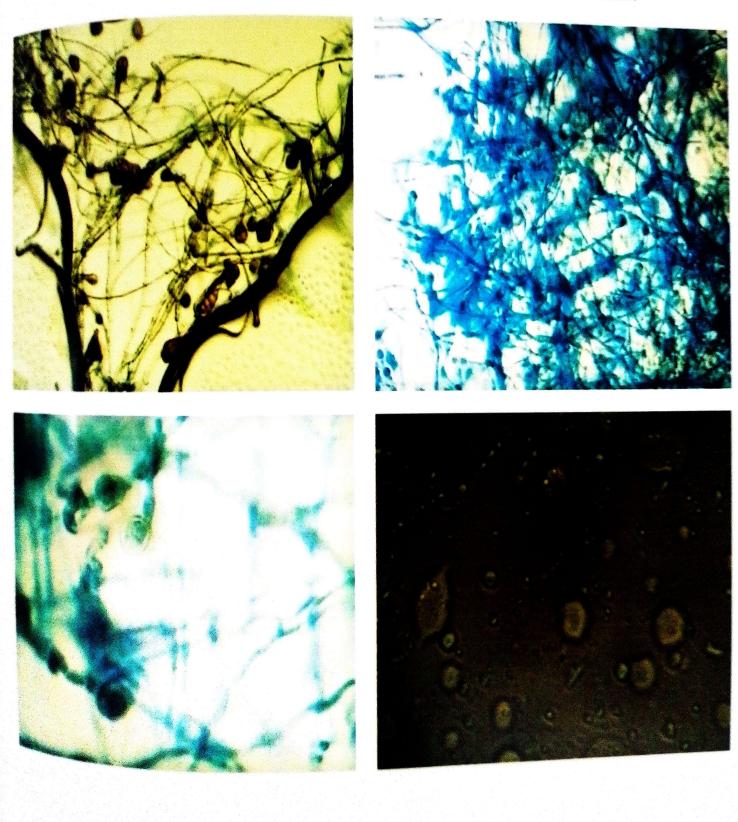




PLATE: 4.3 Morpholigical identification of Ganoderma lucidium



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JABALPUR (M.P.)

SESSION: 2021-2022

DEPARTMENT OF ZOOLOGY

Project Report

On

LIFE STYLE DISEASES-INSOMNIA

Submitted to Dept. of Zoology

Submitted by
Upadhi Tekam
M. Sc. II Semester

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ACKNOWLEDGEMENTS

I would like to extend my sincere gratitude towards principal Dr.Fr.G. Vazhan Arasu and Head, Department of Zoology Dr. Priyanka Sinha, St. Aloysius College (Autonomous) Jabalpur for providing laboratory facilities, extending necessary help and kind support to Carry out this project work.

I am enormously indebted to all my classmate for extending their cooperation and help during the collection of leaf and insect.

Last but not the least I express my gratitude towards my parents and my family for their unconditional love and support during the completion of the project.

Upadhi Tekam M. Sc. II Semester

DECLARATION

I, Upadhi Tekam of class M.Sc. II semester Zoology, St. Aloysius College Jabalpur, M.P. that I have has made project report on Life Style Diseases-Insomnia, at Jabalpur, 2021-22 and prepared this report original by me.

Signature

INTRODUCTION

Lifestyle diseases can be defined as diseases linked with one's lifestyle. These diseases are non-communicable diseases. They are caused by lack of physical which can lead to heart disease, stroke, insomnia, anxiety, obesity, type II workforce and the cost of health care. Treating these non-communicable diseases primary prevention and identify early symptoms of these non-communicable disease. These lifestyle disease are expected to increase throughout the years if people do not improve their lifestyle choices.

INSOMNIA

Insomnia, the inability to sleep adequately. Causes may include poor sleeping conditions, circulatory or brain disorders, a respiratory disorder known as apnea, stress, or other physical or mental disorders. Insomnia is not harmful if it is only occasional; the body is readily restored by a few hours of extra sleep. If, however, it is regular or frequent, insomnia may have harmful effects on other systems and functions of the body. Between 10% and 30% of adults have chronic insomnia, and many more deal with it from time to time. Here's a look at some of the latest research on this condition, its causes, and treatments that may help.

► INSOMNIA STATISTICS

Global sleep deprivation

According to recent studies, 20% of people around the world are sleep deprived.



TYPES OF INSOMNIA

Not all cases of insomnia are identical. People can be affected by insomnia in different ways, and distinguishing between forms of the condition can be useful for both health professionals and people with insomnia.

There are two main types of insomnia:

Short-Term Insomnia

Also known as acute insomnia or adjustment insomnia, this is a brief episode of difficulty sleeping. Short-term insomnia is often caused by a stressful life event, such as the loss of a loved one, a disconcerting medical diagnosis, a pandemic, rebounding from cessation of a drug or marijuana, or a major job or relationship change.

Acute insomnia lasts for less than three months, and symptoms may fade on their own as time passes and a person copes with the stressful incident that gave rise to their sleeping problems. However, short-term insomnia can be persistent and become chronic insomnia.

Short-term insomnia can affect both children and adults. It is more common in women1 than in men, and it can arise during pregnancy as well as menopause.

Chronic Insomnia

Chronic insomnia is a long-term pattern of difficulty sleeping. Insomnia is considered chronic if a person has trouble falling asleep or staying asleep at least three nights per week for three months or longer.

Some people with chronic insomnia have a long history of difficulty sleeping. Inability to get the sleep they need may be persistent or go away and recur with months-long episodes at a time. Chronic insomnia has many potential causes. Like acute insomnia, it can be tied to stressful situations, but it may also be related to irregular sleep schedules, poor sleep hygiene, persistent nightmares, mental health

disorders, underlying physical or neurological problems, medications, a bed partner, and certain other sleep disorders.

Like short-term insomnia, chronic insomnia occurs in people of all ages and has a higher prevalence in women. While the sleep disorder of insomnia is principally classified as either short-term or chronic, there are other terms that may be used to describe insomnia. These terms may be used informally or as a way for researchers to better categorize and analyzes the different ways that insomnia can be experienced.

Sleep Onset Insomnia

Sleep onset insomnia describes difficulty falling asleep at the beginning of the night, or in the case of shift workers, whenever they attempt to initiate sleep. It is associated with the idea of tossing and turning without actually being able to get to sleep. Most people with sleep onset problems can't fall asleep even after spending 20-30 minutes in bed. The inability to fall asleep means that a person with insomnia of this nature has reduced total sleep time and can feel the effects of that lack of sleep the next day.

Sleep Maintenance Insomnia

Sleep maintenance insomnia describes an inability to stay asleep through the night. Most often, this means waking up at least once during the night and struggling to get back to sleep for at least 20-30 minutes. The fragmented sleep associated with poor sleep maintenance means a decrease in both sleep quantity and quality, creating higher chances of daytime sleepiness or sluggishness.

Early Morning Awakening Insomnia

Early morning awakening insomnia involves waking up well before a person wants or plans to in the morning. Some experts view this as a component of sleep maintenance while others consider it separately. Inability to get their desired amount of sleep can impair a person's physical and mental function the next day.

Mixed Insomnia

Though not a formal term, the descriptor mixed insomnia may be applied to people who have a combination of problems related to sleep onset, sleep maintenance, and early morning awakenings. In general, the broader term insomnia can be thought of as describing mixed insomnia because it is common for people to have overlapping sleeping problems. In addition, people with insomnia often find that their symptoms can shift over time, making it hard to classify insomnia strictly among the subtypes of onset, maintenance, and early morning awakenings.

Comorbid Insomnia

In the past, sleeping problems were sometimes labeled as comorbid insomnia or secondary insomnia. This meant that insomnia was believed to arise as a result of another condition such as anxiety, depression, sleep apnea, gastroesophageal reflux disease (GERD), or physical pain.

Contemporary research has generated a deeper understanding of insomnia that recognizes that sleeping problems often have a bidirectional relationship with other health issues. For example, while anxiety may contribute to insomnia, insomnia may trigger or exacerbate anxiety as well. Furthermore, insomnia triggered by another condition does not always go away even after that underlying issue is resolved.

Because of the complexity of these relationships, it becomes difficult to classify insomnia as strictly comorbid or secondary. Similarly, the presence of multiple contributing factors makes identifying a single cause of insomnia challenging for many patients.

For these reasons, insomnia classification systems used in sleep medicine have moved away from this terminology and toward a broader understanding of insomnia

Causes of insomnia

Insomnia can result from a range of physical and psychological factors. Often, the cause is a temporary problem, such as short-term stress. In some other instances, insomnia stems from an underlying medical condition.

Genetics- Scientists are looking at whether the likelihood of having insomnia is something you're born with. They've identified specific areas in our genes that appear to play a role in insomnia symptoms.

Common causes include:

- having jet lag, switching shifts at work, or dealing with any other changes to the body's internal clock
- the room being too hot, cold, or noisy, or the bed being uncomfortable
- caring for someone in the house, if it disrupts sleep
- · getting too little physical exercise
- · having night terrors or bad dreams

using recreational drugs, such as cocaine or ecstasyIn some people, stress or a mental health issue is responsible for insomnia. A person may be experiencing:

- depression
- anxiety
- bipolar disorder
- schizophrenia

Some other health conditions that can limit sleep include:

- restless legs syndrome
- an overactive thyroid
- sleep apnea
- gastrointestinal reflux disease, commonly called GERD
- chronic obstructive pulmonary disease, known as COPD
- · chronic pain

Often, symptoms of another health issue or natural transition cause difficulty sleeping. During menopause, for example, hormonal changes can lead to night sweats, which can interrupt sleep. In people with Alzheimer's disease, changes in the brain disrupt or change sleeping patterns. Also, some people have a rare genetic disorder called fatal familial insomnia, which prevents sleep and can be life threatening.

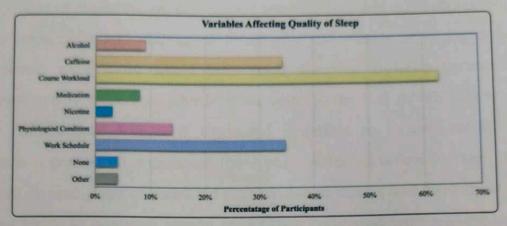


Figure 2. Variables Affecting Quality of Sleep

CONSEQUENCES OF INSOMNIA

Due to its chronicity, insomnia is associated with substantial impairments in an individual's quality of life. In several studies, insomniacs reported decreased quality of life on virtually all dimensions of the 36-item Short Form Health Survey of the Medical Outcomes Study (SF-36), which assesses 8 domains: (1) physical functioning; (2) role limitation due to physical health problems (role physical); (3) bodily pain; (4) general health perceptions; (5) vitality; (6) social functioning; (7) role limitations due to emotional health problems (role emotional); and (8) mental health. One study compared SF-36 results in groups of mild and severe insomnia patients with groups of patients diagnosed with depression or congestive heart failure (CHF). Severe insomnia patients had numerically greater loss of function than patients with CHF in reported pain, emotional effects, and mental health effects. Additionally, insomnia patients also reported more physical problems than patients with depression.

Research has shown that among the daytime consequences of insomnia, the increased occurrence of accidents poses the greatest health risk. Insomniacs are 2.5 to 4.5 times more likely than controls to have an accident. In a sample of 8,625 community respondents in France, Léger et al. reported that 8% of insomniacs and 1% of non-insomniacs had an industrial accident in the past 12 months. Work productivity is also compromised among insomniacs due to work-related problems (ie, higher rates of absenteeism, decreased concentration, and difficulty performing duties). Kuppermann and colleagues found that individuals reporting a current sleep problem were more likely than good sleepers to have decreased job performance and to have been absent from work in the last month due to health problems. Simon and VonKorff evaluated insomnia in a staff-model health maintenance organization population (N=1,962). After adjusting for age, gender, and chronic disease, days of restricted activity due to illness and days spent in bed were about twice as common among insomniacs compared with non-insomniacs. Additionally, mean total health care expenditures were 60% higher in the insomnia group relative to the controls.

Population- and clinic-based studies have demonstrated a high rate of psychiatric comorbidities in patients with chronic insomnia. In fact, insomnia is more frequently associated with psychiatric disorders than any other medical illness. For example, in the Epidemiologic Catchment Area study, 40% of insomniacs had a comorbid psychiatric disorder compared with 16.4% of those with no sleep complaints. Additionally, depression and anxiety are the most common comorbid psychiatric disorders in insomniacs. It has traditionally been assumed that insomnia is secondary to the psychiatric disorder; however, given the chronicity of insomnia, it is possible that in some, if not most, cases the insomnia precedes the psychiatric disorder. In fact, it is possible that insomnia represents a significant risk for the development of a subsequent psychiatric disorder. In a large-scale European population-based study (N=14,915), it was found that insomnia more often preceded rather than followed incident cases of a mood disorder. This effect is even more pronounced for relapses of the mood disorder, where in 56.2% of cases, insomnia symptoms preceded symptoms of a mood disorder relapse. In

contrast, in chronic insomnia patients with a comorbid anxiety disorder, the first occurrence of anxiety or a relapse preceded insomnia in most instances.

To further understand the relation of sleep and psychiatric disorders, several longitudinal studies have examined the evolution of psychiatric disorders among insomnia patients. These studies used follow-up periods ranging from 1 to 40 years, with the majority using a 1- to 3-year follow-up period. In all of these studies, insomnia has been found to confer a substantial risk for the development of a depressive disorder. Typically, the relative risk was approximately 5 (range 2–40), and in all cases it was statistically significant. While some studies also reported an increased risk for anxiety or drug abuse, neither of these was consistently found. Finally, longitudinal studies in subjects with affective disorders show that depressed patients who experience improvements in sleep will also experience a more rapid antidepressant response; while those patients whose insomnia persists will have a short time to relapse. What is clearly needed are clinical trials to assess the impact of insomnia therapy on incidence of depression as well as the time to relapse in depressed patients who are in remission.

The question then arises as to whether insomnia causes depression, vice versa, or both. The close association of insomnia with depression is likely related to common underlying pathophysiological mechanisms for sleep and mood regulation that make the individual vulnerable to both conditions. Data have shown that both the diagnosis of insomnia and the severity of the sleep disturbance are related to overactivation of the hypothalamic-pituitary-adrenal (HPA) axis and the hypersecretion of cortisol.Recent evidence suggests that there may be some neuroendocrine and clinical similarities between insomnia and depression. Corticotropin-releasing factor (CRF) dysregulation has been implicated in the pathogenesis of psychiatric disorders such as depression as well as in the mediation of hyperarousal seen in primary insomnia. This abnormality might represent the common risk factor, and therefore, it is quite possible that both would respond to the same therapeutic intervention corticotropinreleasing hormone antagonists).

Insomnia Medications

A new type of drug is giving doctors another option when it comes to prescription medication to treat insomnia. Or exin receptor antagonists. Some of the latest sleep medications are known as or exin inhibitors. They block the action of a brain chemical that keeps you alert, called or exin. Two of these drugs include:

Lemborexant (Dayvigo)

Suvorexant (Belsomra)

Scientists are studying two other similar drugs in clinical trials.

All sleeping pills come with risks and side effects. With some, you can develop a tolerance, which means it stops working effectively at the recommended dosage, or even feel like you can't fall asleep without them. Some can cause you to walk, eat, or even drive in your sleep. Most can leave you feeling groggy the next day. Doctors usually only prescribe them for the short-term, and you have to stop using them gradually.

Melatonin. Your body makes this hormone as the sun goes down, telling you it's time to get sleepy. A prescription form of melatonin helps you fall asleep by acting like melatonin in your brain. Melatonin supplements are often sold as a treatment for insomnia.

How To Diagnose Insomnia

The requirements for an insomnia diagnosis are continually evolving as researchers learn more about this sleep disorder. According to current criteria, patients must report at least one of the following problems in order to receive an insomnia diagnosis.

- Difficulty falling asleep
- Difficulty remaining asleep during the night
- Repeated instances of waking up earlier than desired
- Feelings of resistance about going to sleep at a reasonable hour
- Difficulty sleeping without help from a parent or caregiver

Additionally, patients must experience one or more of the following daytime impairments after a night of insomnia-affected sleep:

- · Feelings of fatigue or malaise
- Difficulty concentrating, paying attention, recalling, or remembering
- · Impaired performance in social, family, academic, or occupational settings
- Excessive daytime sleepiness
- · Hyperactivity, impulsivity, aggression, and other behavioral problems
- · Decreased energy, motivation, or initiative
- · Higher risk of errors or accidents
- · Concerns or dissatisfaction regarding sleep

These nighttime and daytime symptoms must occur despite adequate opportunity for sleep and a bedroom environment that is conducive to sleep. If symptoms occur at least three times per week for at least three months, the patient will be diagnosed with chronic insomnia. If these benchmarks have not been reached, then the condition is known as short-term or acute insomnia. A third condition known as other insomnia may be diagnosed if the patient does not meet the criteria for short-term insomnia but nonetheless exhibits insomnia symptoms.

The cause of a person's insomnia symptoms is also crucial to their diagnosis. Primary insomnia occurs independently, while secondary insomnia is usually attributed to an underlying medical or psychological condition that causes sleep loss. Primary and secondary insomnia share the same symptoms. However, treatment for secondary insomnia will typically also address the patient's underlying condition.

Prevention

Prevention and treatment of insomnia may require a combination of cognitive behavioral therapy, and lifestyle changes. Among lifestyle practices, going to sleep and waking up at the same time each day can create a steady pattern which may help to prevent insomnia. Avoidance of vigorous exercise and caffeinated drinks a

few hours before going to sleep is recommended, while exercise earlier in the day may be beneficial. Other practices to improve sleep hygiene may include:

- Avoiding or limiting naps
- · Treating pain at bedtime
- Avoiding large meals, beverages, alcohol, and nicotine before bedtime
- Finding soothing ways to relax into sleep, including use of white noise
- Making the bedroom suitable for sleep by keeping it dark, cool, and free of devices, such as clocks, cell phones, or televisions
- Maintain regular exercise
- Try relaxing activities before sleeping

Background

A survey of 1.1 million residents in the United States found that those that reported sleeping about 7 hours per night had the lowest rates of mortality, whereas those that slept for fewer than 6 hours or more than 8 hours had higher mortality rates. Getting 8.5 or more hours of sleep per night was associated with a 15% higher mortality rate. Severe insomnia – sleeping less than 3.5 hours in women and 4.5 hours in men – is associated with a 15% increase in mortality.

With this technique, it is difficult to distinguish lack of sleep caused by a disorder which is also a cause of premature death, versus a disorder which causes a lack of sleep, and the lack of sleep causing premature death. Most of the increase in mortality from severe insomnia was discounted after controlling for associated disorders. After controlling for sleep duration and insomnia, use of sleeping pills was also found to be associated with an increased mortality rate.

The lowest mortality was seen in individuals who slept between six and a half and seven and a half hours per night. Even sleeping only 4.5 hours per night is associated with very little increase in mortality. Thus, mild to moderate insomnia for most people is associated with increased longevity and severe insomnia is associated only with a very small effect on mortality. It is unclear why sleeping longer than 7.5 hours is associated with excess mortality.

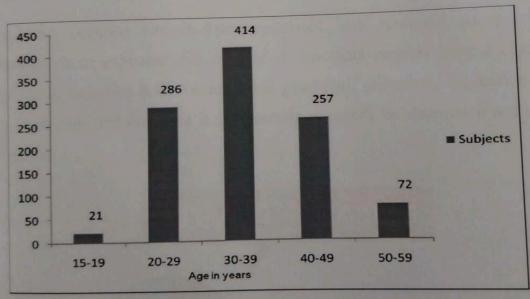
While insomnia is typically transient, some cases are persistent and can last for years. 'Insomnia disorder' should be diagnosed using diagnostic criteria from the American Psychiatric Association's Diagnostic and Statistical Manual (DSM) and/or the International Classification of Sleep Disorders (ICSD). Both have been recently updated. The fifth edition of the DSM (DSM-5) is geared towards primary care and general mental health providers. Criteria for insomnia disorder require that sleep symptoms cause clinically significant distress or impairment(s) in functioning (social, occupational, educational, academic, behavioral, or other) and occur despite adequate opportunity for sleep on at least 3 nights per week for at least 3 months. Diagnosis also requires that symptoms not be primarily linked to other sleep disorders or occur exclusively during the course of another sleep-wake disorder (narcolepsy, breathing-related sleep disorder, circadian rhythm disorder); not be attributable to the physiological effects of a substance; and not be explained by coexisting mental disorders or medical conditions. Dysfunction associated with insomnia disorder includes fatigue, poor cognitive function, mood disturbance, and distress or interference with personal functioning. Both criteria recognize sleeprelated complaint(s) despite adequate opportunity for sleep combined with distress or dysfunction created by the sleep difficulty in their current and previous versions. Until recently, diagnostic criteria classified insomnia as primary or comorbid, depending on the absence or presence of other conditions.

PREVALENCE OF INSOMNIA

Estimates of the prevalence of insomnia depend on the criteria used to define insomnia and more importantly the population studied. A general consensus has developed from population-based studies that approximately 30% of a variety of adult samples drawn from different countries report one or more of the symptoms of insomnia: difficulty initiating sleep, difficulty maintaining sleep, waking up too early, and in some cases, nonrestorative or poor quality of sleep. Conclusions from the NIH State-of-the-Science Conference held in June 2005 indicate that the addition of a diagnostic requirement that includes perceived daytime impairment or distress as a function of the insomnia symptoms results in approximately 10%

prevalence of insomnia. Finally, the application of more stringent diagnostic criteria, such as the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), which includes the additional requirements that insomnia symptoms persist for at least 1 month and do not exclusively occur in the presence of another sleep disorder, mental disorder, or the direct physiological effects of a substance or medical condition, yields current prevalence estimates of approximately 6%.

Several well-identified risk factors for insomnia were reported by the State-of-the-Science Conference in June 2005. Age and gender are the most clearly identified demographic risk factors, with an increased prevalence in women and older adults. While the cause of this increased risk in the elderly is not well defined, it may be due to the partial decline in functionality of sleep control systems that may contribute to insomnia in this older population.

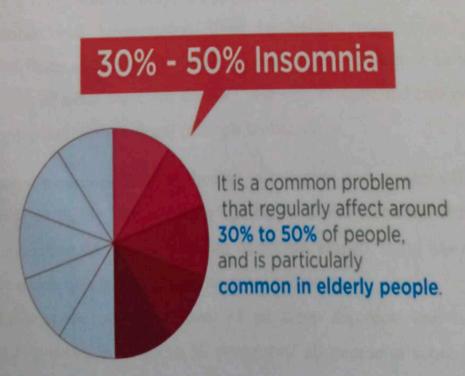


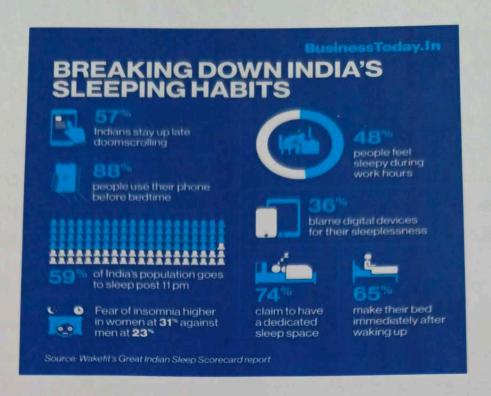
Importantly, the presence of comorbid medical conditions is also a significant contributor to the increased prevalence of insomnia in the elderly. Additionally, in women, insomnia is more prevalent with both the onset of menses and menopause. Comorbid medical disorders, psychiatric disorders, and working night or rotating shifts all represent significant risks for insomnia. It is important to recognize that these factors do not independently cause insomnia, but rather they are precipitants of insomnia in individuals predisposed to this disorder. In fact, chronic illnesses

are a significant risk for insomnia. It is estimated that the majority of people with insomnia (approximately 75%–90%) have an increased risk for comorbid medical disorders, such as conditions causing hypoxemia and dyspnea, gastroesophageal reflux disease, pain conditions, and neurodegenerative diseases. Importantly, a variety of primary sleep disorders as well as circadian rhythm disorders are frequently comorbid with and often lead to insomnia.

Among the primary sleep disorders, restless legs syndrome (RLS), periodic limb movement disorders (PLMD), and sleep-related breathing disorders (snoring, dyspnea, sleep apnea) often present with an insomnia symptom. This is especially true among the elderly. Among younger individuals, difficulty falling asleep is often associated with a phase delay syndrome. However, in the elderly, phase advance syndrome results in reports of difficulty initiating sleep, maintaining sleep, and experiencing early morning awakenings.

The most common comorbidities associated with insomnia are psychiatric disorders. It is estimated that 40% of all insomnia patients have a coexisting psychiatric condition. Among these psychiatric disorders, depression is the most common, and insomnia is a diagnostic symptom for depressive and anxiety disorders.





Insomnia Facts

Insomnia or poor sleep health is not regarded as a public health crisis but many experts believe that it should be treated as one. The burden of insomnia has been grossly underestimated because the focus of attention is restricted towards few other conditions that contribute to non-communicable diseases. While diets and obesity have a huge role to play, the importance of sleep to general health and wellbeing cannot be underestimated. Sleep deprivation, insomnia and other sleep disorders contribute significantly to the global burden of disease as they greatly increase the risk of cardiovascular disease, diabetes, obesity and cancer, as well as the risk of injury and violent death through human error.

- Increased awareness on the disease is essential and greater efforts need to be made to tackle the paucity of global data and insomnia statistics. What was once thought of as a problem of the developed world has now become just as much of a burden in developing countries.
- Insomnia is the most prevalent of all sleep disorders and is believed to affect anywhere from 30 to 50 percent of all people at some point in their lifetime.

- Long-term sleep deprivation can cause severe health problems and compromise your quality of life. It is estimated that chronic insomnia affects as much as ten percent of these individuals.
- Insomnia affects one out of four people and it isn't gender or age specific. Insomnia in children may be uncommon but it is certainly not unheard of. With increased examination and performance stress these figures are only rising. The condition is increasingly prevalent as you age with 40 to 60 percent of all individuals above the age of 60 suffering from insomnia.

Insomnia in Children

- Insomnia is twice as likely to affect women as compared to men. Chronic insomnia greatly increases the risk of various non-communicable diseases.
 There were 57 million deaths in 2008, with 58% of these being attributed to chronic (non-communicable) diseases. These numbers could be significantly reduced if sleep disorders were addressed.
- Studies have shown that the risk of strokes, hypertension, arteriosclerosis, obesity, diabetes and depression are much higher in patients suffering from insomnia.
- Substance abuse like smoking, alcoholism and drugs are more likely to develop in individuals suffering from insomnia.
- Fatigue and drowsiness cause around 100,000 traffic accidents a year, claiming as many as 1,500 lives and leaving over 70,000 injured. Now take into account that these figures are limited to the United States which accounts for just 5 percent of global population.
- Researchers have found that insomnia isn't just a problem in humans but
 can affect our pets as well and pests too! Fruit flies were the test subjects in
 the study and they were found to display symptoms very similar to those
 seen in humans such as impaired balance and weight gain.
- Most of us tend to think that following a regular routine on weekdays will suffice to keep us healthy. Unfortunately, those wild weekends and late night parties also take their toll. Studies suggest that people with different

sleep schedules on weekdays and weekends are more likely to suffer from sleep deprivation and obesity.

There is no miracle cure or medication for insomnia that can solve the problem. Sleeping pills do not cure insomnia and can even contribute to the development of chronic insomnia. Cognitive behavioral therapy is the only strategy with a proven track record.

Sleeping Pills

Fatal familial insomnia should not be confused with acute or chronic insomnia. It's a much more serious condition that is caused as a result of a genetic disease. This disease prevents the victim from falling asleep and the duration of sleeplessness keeps increasing until the lack of sleep turns fatal. Thankfully, this is not a common condition.

OBJECTIVE -

To perform a survey on awareness of lifestyle disease (insomnia) among the local people of a particular area (Jabalpur):

Requirements – questionnaire paper, pen, cardboard, etc.

Methodology -Survey methodology as a scientific field seeks to identify principles about the sample design, data collection instruments, statistical adjustment of data, and data processing, and final data analysis that can create systematic and random survey errors.

There are many methods to conduct a survey ,here I have used the questionnaire method for collecting data .

<u>Analysis and interpretation of data</u>-The method used for surveying is questionnaire, 17 questions are included in this questionnaire. the survey was conducted on 100 people of a local area in Jabalpur.

Observation:

The responses (majority) to questions are written below:

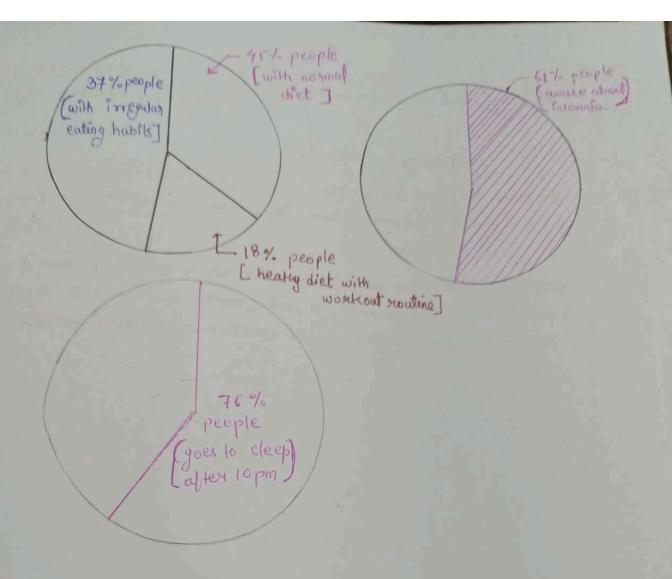
S.no	Response to question(majority)	No. of other reaponses	No. of responses(majority)
1.	Normal healthy meals	55	45
2.	yes	42	58
3	Good	60	30
4	yes	50	50
5.	6-7 hours	64	36
6.	Regular sleep	46	54
7.	After 10pm	24	76
8.		42	58
9.	Rarely	30	60
0.	Yes	26	64
1.	yes	46	54
12.	sometimes	48	52
13.	Rarely	60	30
14.	Sometimes	36	64
15.	Yes Prefer going to calm or	10	54
16	peaceful environment	30	70
17.	No Yes	28	72

ST ALOYSIUS COLLEGE JABALPUR

SURVEY ON LIFESTYLE DISEASE -INSOMNIA

CIVELES TYLE DI	SEASE –INSOMNIA					
Age- >40	<50 □ ∠40 □					
Gender-						
Q.1 What is your daily lifestyle is:						
normal healthy meals	irregular eating habits					
healthy diet and regular excercising						
Q.2 Are you aware of the problems that could arise du	re you aware of the problems that could arise due unhealthy lifestyle?					
Yes	No 🗆					
Q.3 In general, how is your current health:						
Excellent	Good Poor Poor					
Q.4 Do you know about any sleeping disorders like insomnia?						
Yes	No 🗆					
Q.5How many hours do you sleep?						
6-7 🗆 7-9 🗀	5-6 🗌 8-10 🔲					
Q.6How would you describe your sleeping pattern:						
Irregular sleep pattern	regular sleep pattern					
Q. 7 What time do you go to bed at night?						
Before 10 pm	After10 pm					
Q.8 Do you have trouble sleeping once in bed?	AL YOUR T					
Never	Rarely Always A					
Q.9Have you noticed any change in your sleeping pattern?						
	No					
Yes	n the morning?					
Q.10 If yes do you feel refreshed upon waking up in the morning?						
Yes	No L					
Q.11Do you have trouble concentrating on something?						
	No Somewine					
Ves	yday chores?					
0.12 Do you often feel tired and unable to do						

Yes	NO 🗆	Randy [
Q.13 Do you feel sleepy during day time?		4			
Yes	No 🗆	Sometimes 17			
Q.14 Have you been in a stressful situation lately	7				
Yes	No				
Q.15 How did deal with a stress?					
I talk to family or friends	I prefer going to a calm of	r peaceful environment			
I like to travel	the option is not availab				
Q.16Do you take any medicine, which type (optional)?					
Yes -	No 🗆				
Q.16 So do you think change in sleeping patterns can cause serious problems?					
Yes	No .				



<u>Result</u> – On the basis of the survey conducted following results are obtained:

- 1. Majority (45%) of people have a normal diet and 18% people have a healthy diet and workout routine.
- 2. Majority (61%)of people are aware about insomnia.
- 3. 76% people sleep after 10 pm which suggests that they have higher chances of becoming insomniac.
- 4. The above table shows that 58% people sometimes find it hard to sleep.
- 5. 54% people sometimes have trouble concentrating.
- 6. The above observation shows that 64% people have been in stressful situation, which in longterm results in sleeping disorders.
- 7. 54% people found it hand to concentrate on focus on something (work).

Conclusion-In conclusion majority of people are aware about insomnia or have suffered from any type of insomnia.

It was observed that lifestyle choices does affect health of an individual.

Recommendation – For a healthy life and a healthy society it is recommended that people of the country are educated and aware to make better choices to live a healthy life.

It is recommended that people should be self-aware and indulge in a healthy lifestyle .if someone is suffering from any disease, they should consult a professional . Lifestyle diseases if taken lightly could lead to dangerous disorders .

REFERENCE

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Elucidation of Oligomeric characteristics of MarR family protein of Mycobacterium tuberculosis

A Dissertation submitted to St. Aloysius College in partial fulfilment of the requirement for the award of the degree of

Master of Science

In

Biotechnology

By

JYOTSANA PATEL

Roll No: 4202777



Department of Biotechnology St. Aloysius College Jabalpur-482001

Under the supervision of Dr. Charu Sharma Senior Principal Scientist

CSIR-IMTECH Chandigarh



CSIR-IMTECH 2022

DECLARATION

I Jyotsana Patel, student of St. Aloysius College, Jabalpur (M.P.), have completed the project titled "Elucidation of oligomeric characteristics of MarR family protein of Mycobacterium tuberculosis" for the award of Degree of Master of Science in Biotechnology for academic session 2021-2022. The information given in this project is true to the best of my knowledge.

Jyotsana Patel



ST. ALOYSIUS' COLLEGE

(AUTONOMOUS)

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CERTIFICATE

This is to certify that the work entitled, "Elucidation of oligomeric characteristics of MarR family protein of Mycobacterium tuberculosis" in CSIR-IMTECH, Chandigarh submitted by Ms. JYOTSANA PATEL partial fulfilment for the award of degree of Master of Science in Biotechnology, St. Aloysius College, Jabalpur Madhya Pradesh has been carried out under the supervision of Dr. Charu Sharma, Senior Principal Scientist of CSIR-IMTECH, Chandigarh.

Q

Dr. L.K. Pandey

Professor and Head of the Department

Department of Biotechnology

Dated: 30/06/22



सीएसआईआर – सूक्ष्मजीव प्रौद्योगिकी संस्थान

बेक्टर ३० - ए. चन्डीवड़ - १६० ०३८ (चनस)

CSIR-INSTITUTE OF MICROBIAL TECHNOLOGY

IA CONSTITUENT ESTABLISHMENT OF CISING Sector 39-A. Chambiganh-160 036 (INDIA)

CERTIFICATE

This is to certify that the thesis entitled, "Elucidation of Oligomeric characteristics of MarR family protein of Mycobacterium tuberculosis" submitted by Ms. Jyotsana Patel (4202777) in partial fulfillment for the award of degree of Master of Science in Biotechnology, St. Aloysius College, Jabalpur, Madhya Pradesh has been carried out from 11.02.2022 to 21.06.2022 under my supervision at CSIR-IMTech, Chandigarh. This work is original and has not been submitted in part or full to any other university or institute for the award of this or any other degree or diploma.

Charu Sharma)

Senior Principal Scientist

CSIR-IMTech

Acknowledgement

Foremost, praises & thanks to the Almighty God because without his graces and blessings. I won't be here, doing things I want.

First I would like to thank my family, who support my decision of coming so far from them, the love which I get from them, have no limits They have always there for me, my mother (my superwoman), my father and his lessons, my elder sister cum my best friend and my little siblings cum entertainers to me, thanks a lot.

Next I would like to thank my HOD Dr. LX Pandey for his support and immeasurable guidance. A picture I draw of a teacher in my mind, he's totally fit in it. I'm always thankful to you for making such a comfort zone, which allow us to talk to you any time, in any circumstances. And thanks to all the Professors of Biotechnology department for their precious guidance.

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At last I wanted to mention my friends, the whole IMTech girl's gang who is like a family to me and were always there to cheer me with full support when I need it. All those Sundays & fun days we spent together made this Imtech journey a memorable journey of my life.

Jyotsana Patel

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Key to Abbreviations

Miscellaneous

TB	Tuberculosis	
Mtb	Mycobacterium tuberculosis	
MDR-TB	Multiple Drug Resistant Tuberculosis	
XDR-TB	Extensively Drug Resistant Tuberculosis	
TDR-TB	Total Drug Resistant Tuberculosis	
MTBC	Mtb Complex	
WHO	World Health Organization	
His	Histidine	
HIV	Human Immunodeficiency Virus	
AIDS	Acquired Immune Deficiency Syndrome	
TLR	Toll Like Receptor	
NTD	N-Terminal Domain	
CTD	C-Terminal Domain	
NOD	Nucleotide-binding Oligomerization Domain	
MR	Mannose Receptor	
NLR	NOD Like Receptor	
CLRs	C-type Lectin Receptors	
SR	Scavenger Receptor	
Sp	Surfactant Protein	
LAM	Lipoarabinomannan	
LAMP	Loop-mediated Isothermal Amplification	
NAAT	Nucleic Acid Amplification Test	
PCR	Polymerase Chain Reaction	
BCG	Bacillus Calmette-Guerin	
LPA	Line Probe Assay	

Weights and Measures

°C	Degree Centigrade
bp, kb	Base pair, Kilo base
ng, μg, mg, g	Nanograms, Micrograms, Milligrams, Grams
μL, ml, L	Microliter, Millilitre, Litre
μM, mM, M, N	Micromolar, Millimolar, Molar, Normal
Sec, min, h	Second, Minutes, Hour
kDa	Kilo Dalton
OD	Optical Density
rpm	Revolutions per minute

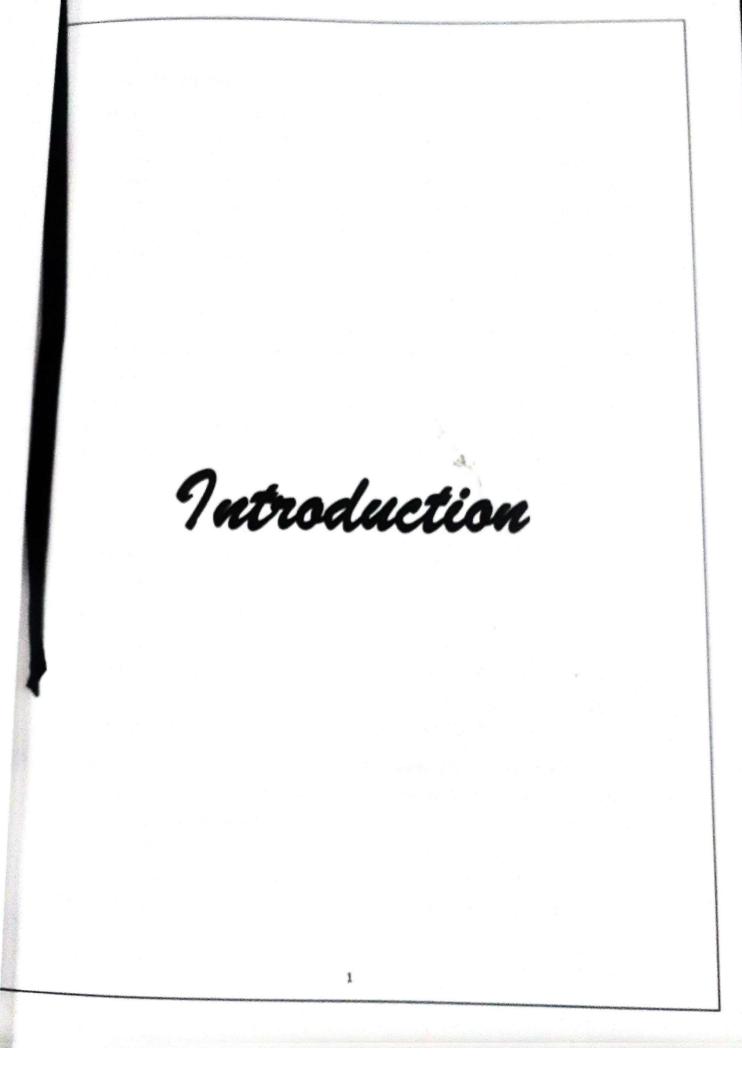
Techniques

SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide	
	Gel Electrophoresis	
Affinity Chromatography	Ni-NTA	
FPLC	Fast Protein Liquid Chromatography	

Chemicals

Ammonium Persulfate	
Bovine Serum Albumin	
Isopropyl-β-D-thiogalactopyranoside	
N, N, N, N-tetramethyl-ethane-1,2-diamine	
Nickel-Nitriloacetic acid Agarose	
Sodium Dodecyl Sulphate	
β-Mercaptoethanol	
Dithiothreitol	

PBS	Phosphate Buffered Saline
PBST	Phosphate Buffered Saline-Tween 20
MgCl ₂	Magnesium Chloride
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium Bromide
Kan	Kanamycin
Cam	Chloramphenicol
Amp	Ampicillin
CaCl ₂	Calcium Chloride
PMSF	Phenyl methyl sulfonyl fluoride
DDW	Double Distilled Water



1. INTRODUCTION

Tuberculosis is one of the deadliest disease caused by Mycobacterium tuberculosis (Mtb). Worldwide, TB is the popular most cause of death holding 13th position and reported to be second topmost infectious killer after COVID-19 (above HIV/AIDS). According to WHO, 2021 (https://www.who.int/) one fourth of the world's population, i.e., around 2 million people are suffering from TB infection, while more than 10 million are in the capture of active TB disease yearly. In 2019, 1.2 million children fell ill with TB globally and 465,000 people fell ill with drug-resistant TB (https://www.cdc.gov/). Although the treatment with anti-microbial drugs kill majority of pathogenic bacilli within 1-2 weeks, however, it is recommended to continue multi-drug therapy for at least 6 to 7 months as per directly-observed treatment, short course (DOTS). This extended therapy minimizes the survival of intracellular pathogen within the cell and inhibits the reactivation of infection. Several studies indicated that the dormant bacteria encounter intracellular harsh microenvironments like low oxygen (Hypoxia), nutrition limitation or starvation and pH stress (Deb et al., 2009; Flentie et al., 2016). These amazing versatility of host-induced stresses induce the emergence of stress resistant heterogeneous pathogenic subpopulations of the bug and consequently leads to metabolic advancement and survival enhancement (Prosser et al., 2017). Even in the dormant state Mtb can get reactivated under favourable conditions and starts replication that results in active TB (Veatch and Kaushal, 2018). These properties of Mtb makes it famous for understanding the underlying mechanism of its defence, so that one can come to other favourable conclusions aiding to its effective treatment.

The extraordinary adaptive capability of Mtb to overcome the multiple environmental stresses inside the macrophages makes it a very strong pathogen. Also, the multiple drug resistance to the already existing drug regimen is one of the biggest obstacles in the way of eradication of TB. Therefore, there is a need for new antibiotic and also for new drug targets to deal with this crisis as well as to avoid cross resistance of currently use therapies (Winglee *et al.*, 2015).

Multiple antibiotic resistance Regulator (MarR) proteins are a great attraction for drug evaluation in relevance to their critical role in antibiotic resistance, virulence, cell viability, survival and response to treatment with anti TB drugs (Zheng et al., 2019). MarR system is conserved in human pathogenic bacteria and is reported in Mtb as well. MarR is involved in drug resistance and intracellular survival of Mtb (McDermott et al., 1998). The proteins of MarR family respond to wide variety of stresses by modulating the expression of genes for the

survival of the pathogen. MarR homologs are also reported to have a role in regulating virulence genes of pathogenic bacteria because of their ability to respond to the sudden environmental changes.

The basic structural organization of a MarR proteins consist of a conserved winged helix-turn-helix motif (Han et al., 2019). This wHTH motif binds and recognize palindromic sequences of DNA in a dimeric form (Zhen et al., 2019). Mostly MarR proteins work as sensors to modulate the expression of stress responsive genes. Based on the sequence similarity, eight proteins of Mtb belong to MarR family. MarR homologs in Mtb are namely Rv0042c, Rv0880 (resistance to bedaquiline), Rv2011c, Rv1049 (MosR), Rv2327, Rv0737, Rv2887 and Rv1404 (regulate acid stress). All of these proteins are poorly studied and came into light very recently. According to the sequence analysis, no specific DNA binding motif has been found in case of Mtb and only 60–122 amino acid residues of MarR protein are critical for binding DNA sequences.

Here we have focused on one of the possible transcriptional regulators of MarR family of Mtb, i.e., Rv0042c which has been found to be overexpressed in granuloma site of a human lung of a MDR TB patient and thought to be involved in drug resistance (Rachman 2004, Movahedzadeh et al., 2004). Rv0042c was reported to be indirectly regulated by the DNA binding domain (PhoPC) of response regulator PhoP (Wang et al., 2007). In 2015, the genome wide transcription regulations for Rv0042c have been studied using Chip-Seq (Minch et al., 2015). Besides, whole genome sequencing (WGS) of the linezolid-resistant mutants exhibited intergenic point mutation (G to T) in the Rv0042c-Rv0043c region (Pi et al., 2019). In sum, Rv0042c protein is relatively an unstudied protein. Therefore, it was decided to elucidate the structural characteristics of Rv0042c in vitro in order to understand its biological behaviour in vivo. In this regard, the oligomeric behaviour of Rv0042c under oxidative and reductive stress conditions in vitro have been taken up.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Introduction to Tuberculosis:

Tuberculosis (TB) is one of the deadliest, multisystemic, contagious, air-borne infectious disease which is caused due to the infection of gram positive bacterium *Mycobacterium tuberculosis* (Mtb). Worldwide TB is one of the top three infectious diseases in the world. World Health Organization (WHO) data shows that 1.5 million people died from TB in 2020 (WHO Report 2020). TB is also the 13th leading cause of death globally. As of now, it's the second main infectious cause of death, after COVID-19. TB is the most common in developing countries, but in reference to the Centre for Disease Control and Prevention (CDC Report of 2020), over 7,000 cases of TB were also registered in the United States in 2020.

In year 1689, Richard Mortan confirmed that pulmonary form of Mtb was linked with pathology in the past but due to varying symptoms, until 1820 TB was not recognized as a single disease. Finally in 1832, J. L Schonlein was the first to assign a name 'Tuberculosis' to these conditions (Ajaz et al., 2021).

2.2 Causative agent of Tuberculosis:

TB caused by variety of species of Gram positive bacteria known as Mtb or tubercle bacilli. Robert Koch identified the bacillus, Mtb as the causative pathogen of tuberculosis on 24th March 1882. Later on in 1905 he was awarded the Nobel Prize in physiology and medicine for the same (Schultz *et al.*, 2011). Mycobacteria is a small non spore forming, non-capsulated, aerobic, non-motile, rod-shaped, gram positive bacillus bacteria. It is an acid-fast bacilli having lipid rich wax like cell wall and one of the most recalcitrant bacteria to treat. Most common staining technique is Ziehl Neelsen technique used for detection of acid-fast bacteria (Singhal *et al.*, 2015). Mtb takes long time (almost 24 h) for division and replication.

2.3 Mycobacterium tuberculosis complex (MTBC):

The causative agent of TB is Mtb. The Mtb complex (MTBC) includes a nearly related species that cause tuberculosis in both animals and humans. The MTBC includes four TB causing mycobacteria: M. bovis, M. africanum, M. canetti, and M. microti. M. africanum which is not frequent, but in Africa it is a major cause of TB (Forrellad et al., 2013). M. bovis was once considered as a common cause of TB. M. canetti infection is rare and seems limited to Horn of Africa. M. microti is also rare and observed only in hosts with weak immunity.

2.4 Mycobacterium tuberculosis (Mtb):

Mtb is a thin straight rod bacterium of size $0.4 \times 3 \mu m$ and grow either singly or in pairs or small clumps. The complex lipid of cell wall makes it difficult to be stained by common Gram's Method. Mtb efficiently retains staining dyes as this is not removed even after acidic treatment, therefore, it is classified as an acid-fast bacillus and stained by acid-fast staining using Ziehl-Neelsen technique (Pfyffer 2015).

2.5 Antigenic Structure of Mtb:

Mycobacteria has many unique immunoreactive substances, present as cell wall components. There are three types of antigens present in Mycobacteria, i.e., ones that are actively secreted outside of cell, Ones that are bound to cell wall, and ones which are present only in cytoplasm. The secreted and cell wall bound antigens are the first antigens to be encountered by immune system upon infection and are thus likely the key players for activating immune response and pathogenicity. Cell wall (insoluble) antigens are present on the cell wall and the basic structure of cell wall is typically of Gram-positive bacteria. The innermost cytoplasmic membrane is surrounded with a thick peptidoglycan layer without any outer membrane. Other components of cell are lipids, proteins and polysaccharides. The lipids constitute 60% of cell wall weight and contribute to several biological properties. Mtb has a different lipid components that helps in intracellular survival and also in establishing the infection (Kuang et al., 2022). Lipids of the cell wall attributes to the acid-fastness of bacteria and immunogenicity. Coming to mycobacterial cell envelope, it consists of four main layers: (i) the peptidoglycan (murein) containing plasma membrane or inner membrane (IM). These peptidoglycans are covalently linked with the polysaccharides (arabinogalactan) whose terminal ends are esterified by mycolic acids. Peptidoglycans help in maintaining cell's shape and rigidity. (ii) The peptidoglycan-arabinogalactan complex (AGP), composed of polysaccharides existed externally to peptidoglycan layer. (iii) Outer membrane (OM) or 'mycomembrane', which is covalently linked to AGP via the mycolic acids, a principal constituent of cell wall composed of long chains of α -alkyl and β -hydroxy fatty acids attached to the terminal arabinose units of arabinogalactan and (iv) the outermost capsule (Kalscheuer et al., 2019). Alpha-glucan is the major constituent of the outermost capsule, it has comparatively lesser population of arabinomannan and mannan, proteins and lipids. The major Mtb capsular components mediate interactions with phagocytes that favour bacterial survival. Cytoplasmic Antigens (Ag) includes Ag5, Ag6, Ag14, Ag19, Ag32, Ag38 and Ag60. All these antigen are protein in nature except Ag60 that comprises lipopolysaccharide and protein as a complex.

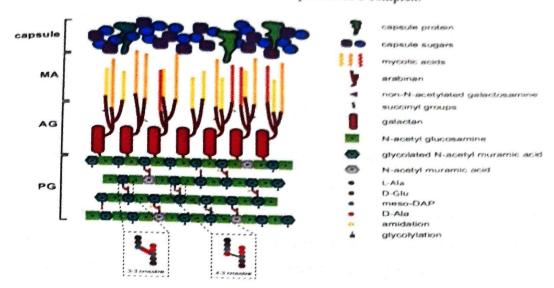
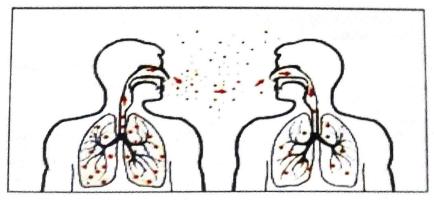


Figure RL1: Cell wall components of Mtb (https://www.semanticscholar.org).

2.6 Transmission of Tuberculosis:

TB is an airborne pathogen that easily transfer from one person to another. The source of infection is usually a patient who is already infected and has an active pulmonary TB. Coughing, speaking or sneezing of the infected patient produces tiny infectious aerosols termed as droplet nuclei of size 1 to 5 microns in diameter (Patterson *et al.*, 2019). Spread of these particles generally depends upon the surrounding environment.



Red dots are droplets containing TB bacteria that are expelled by an infected person when talking, sneezing or coughing and are inhaled by someone closeby and enter their lungs.

Figure RL2: Image showing Transmission of TB pathogen from one person to another through air droplets (https://www.slideshare.net).

Table RL1: Key Factors related of Transmission of Mtb (https://www.cdc.gov/tb/education).

Factor	Description Susceptibility (immune status) of the exposed individual	
Susceptibility		
Infectiousness	Infectiousness of the person with TB disease is directly related to the number of tubercle bacilli that he or she expels into the air. Persons who expel many tubercle bacilli are more infectious than patients who expel few or no bacilli	
Environment	Environmental factors that affect the concentration of M. tuberculosis organisms	
xposure	Proximity, frequency, and duration of exposure	

The rate of infection of a healthy person is directly related to the number of tubercle bacilli that are inhaled or present in the air while contacting a diseased person. Persons with coughing and sneezing symptoms are more infectious in comparison to others (Maher et al., 2009).

The initial infection is referred as primary TB and post-primary or while secondary infection is reinfection of the subsequent disease in a previously treated person, source can be exogenous or by reactivation of a primary infection. Pathological characters of both these infections are quite different as mentioned below in a table:

Table RL2: Primary and Post-primary TB characteristics (Cambier et al., 2014).

Characteristics	Primary	Post-primary
Site	Any part of the lung	Apical region
Local lesion	Small	Large
Cavity formation	Rare	Frequent
Lymphatic involvement	Yes	Minimal
Infectivity	Uncommon	Usual
Tuberculin reactivity	Negative (initially)	Positive
Local spread	Uncommon	Frequent

2.7 Diagnosis of TB:

For diagnosis of tuberculosis, there are many different non-molecular and molecular methods.

2.7.1 Non-Molecular Techniques-

Microscopy is commonly used and the primary TB diagnostic test. It includes the microscopic examination of sputum for acid-fast bacilli (AFB) which is less time consuming, costly, less sensitive and poorly specific especially in patients carrying HIV infection and children. Culturing involves Lowenstein-Jensen, Middlebrooks's 7H9 broth and Middlebrooks's 7H10 and 7H11 agar media. These media provide all the essential nutrition and environmental conditions needed by the bacteria (Foulds et al., 1998). Animal inoculation includes the use of many animal species for TB infection in animal models such as rabbits, guinea pigs, mice and non-human primates. Each of these model has similar features of human TB, either that be clinical signs, pathology, infection burden, progression of the disease or immunology.

But these techniques delayed the process being slow, time consuming, expansive to maintain the infrastructures and well-trained workers. This delay in diagnosis is a major drawback as treatment is dependent on diagnosis and thus gets delayed. Therefore, there is an urgent need for novel quick method with accurate diagnosis.

2.7.2 Molecular Techniques:

As the time is advancing new diagnostic methods are coming into light. Along with the advancement of molecular techniques, different types of tests are available for example; nucleic amplification tests (NAATs) that includes polymerase chain reaction (PCR), real-time PCR, and loop-mediated isothermal amplification (LAMP) (Ghiasi et al., 2015). The most popular test for the diagnosis of TB that is recommended by the WHO is nucleic acid amplification (NAATs).

2.7.3 Rapid Molecular testing of TB:

WHO currently approved different Molecular tests that are: Loop-mediated isothermal amplification test (TB-LAMP) which is commercially developed by Eiken Chemical, Tokyo, Japan; MTB Plus and MTBRIF Dx tests (Molbio Diagnostics, Goa, India) (Vijayalakshmi et al., 2019); Xpert MTB/RIF and Xpert MTB/RIF Ultra assays developed by Cepheid, Sunnyvale, USA which simultaneously pinpoint tuberculosis; Truenat MTB and lateral flow urine lipoarabinomannan assay (LF-LAM) (Bjerrum et al., 2019).

And initially the most recent tests commend by WHO is Truenat (MTB test) (Sharma et al., 2021) or Xpert (MTB/RIF or MTB/RIF Ultra) for fast diagnosis purposes in pulmonary TB. The technique called as NAAT (Nucleic Acid Amplification Tests) are cartridge based technique which detects the presence of TB DNA with common mutations, generally associated with rpoB gene along with RIF resistance within 2 h.

2.8 Treatment of TB:

The patients infected with TB can be cured if the right medication is given at the right time. Treatment given is dependent on the age group of patients as well as different health conditions, types of TB (latent or active) and the area of infective organs (brain, lungs & kidney). TB treatment is often compounded by the issues related to drug resistance, dormancy and persistence. The extensive use of rifampicin and streptomycin was noticed in early 20th century. The first line anti-tuberculosis therapy constitute the four drugs with bactericidal and bacteriostatic activity against TB. These four drugs are Ethambutol (E), Isoniazid (H), Rifampicin (R) and Pyrazinamide (Z) (Shi et al., 2007). Two of the bactericidal core drugs rapidly kill bacteria which reduces infectiousness and slowing down the disease progression. In the events of documented resistance or toxicities, these drugs should be replaced by other drugs with similar efficacies.

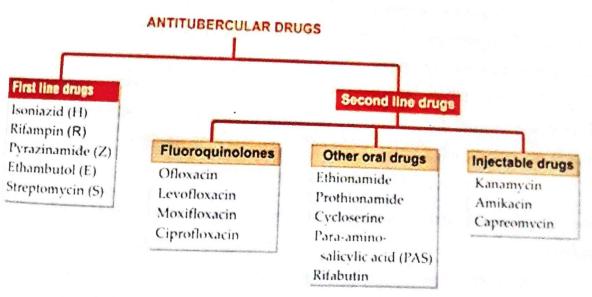


Figure RL3: Examples of Anti-TB first and second line drugs (https://www.jaypeedigital.com).

2.8.1 Treatment of Drug Susceptible TB:

Drug susceptibility of Mtb can either be determined by metabolic inhibition in a medium having anti-tuberculosis drug or by spotting growth or by detecting mutated genes at molecular level. It is also explained as the growth inhibition of pathogen due to drug activity by different means: 1) in vitro growth in drug-free and drug-containing media; 2) metabolic activity detection or measurement 3) mycobacteriophage lysis and 4) analyse the genetic mutations (Kim 2005). In a standard treatment for active TB, a 6 month course of combination of four anti-TB drugs is used. Isoniazid and rifampicin are of prime importance in this treatment, with ethambutol and pyrazinamide given in the first 2 months of treatment (Seid et al., 2018). However, there are chances of failure of treatment if one does not stick to long duration of treatment that will adverse the condition by emerging drug resistance TB treatment.

2.8.2 Treatment of Drug resistance TB:

Despite the availability of effective medication drug-resistant tuberculosis (TB) has posed a great challenge to the people, communities and global TB control society. Along with affecting the patient, Drug resistant TB impose a tremendous burden on overextended public health system and attack the resources which are already limited. Drug-resistant tuberculosis (TB) has emerged as a major contributor to AMR worldwide and threatens the lives of the patients.

Drug resistance is divided into two categories. First is **Primary resistance** when the Mtb infected person has already developed a resistant to some drug. **Secondary resistance** when the drug-resistant organisms which was dormant earlier due to drug treatment emerge back during treatment. This type of resistance occur only when there is a lack of continuation to the medication procedure by the patient. Non-adherence to one drug will generate resistance. In case of adults, the main reason of drug resistant TB recurrence of TB disease, exposure to some drug resistant TB patient or emergence of a co-infection like HIV.

In this era the inception and spreading of Multidrug resistant tuberculosis (MDR-TB) is a very serious outcome of uncurbed drug resistance. WHO defines MDR strain as the strain for which rifampicin and isoniazid shows least effect. MDR-TB requires an extended and high-cost second-line treatment for at least eighteen months as compared to drug-susceptible isolates (Iseman et al., 1993). There cases where first line anti-TB drug i.e. isoniazid and rifampicin is ineffective has increased. The second-line anti-tuberculosis drugs include Fluoroquinolones and Aminoglycosides (Kanamycin, Capreomycin, and Amikacin). With increased cases of

MDR-TB, in vitro testing determines the potency of newer agents. Another challenging condition is extensively drug resistant-tuberculosis (XDR-TB), which can be explained as resistance to first-line agents (rifampicin and isoniazid), any fluoroquinolone and other injectable second-line agents (Amikacin, Kanamycin and Capreomycin). The resistance to all the tested drugs is known as Total Drug Resistant TB (TDR-TB) (Cohen et al., 2014).

2.8.3 Vaccine for TB:

Defence against Tuberculosis termed as Immunotherapy, was proposed by Robert Koch in 1890. Until now, the one and only effective vaccine for tuberculosis in use is the Bacillus Calmette-Guérin (BCG) vaccine. It was used for the first time in 1921 on humans (Soysal et al., 2005). The side effects of BCG vaccine is experienced by almost 3 out of every 10,000 vaccinated people. This vaccine is not given to immuno-compromised personals. Although BCG immunization come up with a fair and effective protection for small children and infants, (including defence against TB meningitis and miliary TB), its efficacy in adults is fluctuating, ranging from 0% to 80%. (Luca et al., 2013). The call for advancement of TB immunotherapy increases day by day because the disease has become increasingly drug-resistant.

BCG strain was isolated by sub culturing a virulent strain of *M. bovis* for about 239 times for period of 13 years on glycerine potato medium. The strain is not virulent to humans but it's capability to induce an immune response is retained. BCG has been widely administered to >3 billion people under the WHO immunization programme (Trunz *et al.*, 2006) with an outstanding safety record (Casanova *et al.*, 1996). BCG has been found protective for extra pulmonary TB and TB meningitis. The vaccine is said to be 60% effective in dealing against common pulmonary form of TB among adolescents and adult population which forms the age group with higher risks for the development of pulmonary active TB disease (Moliva *et al.*, 2017). The efficiency of BCG vaccine dwindles with age.

The immunity provided by the BCG vaccine depends upon the immune response generated by mycobacteria at the site of infection (Dijkman *et al.*, 2019). When BCG vaccine is directly injected into the mucosa of lungs, it induces a local immune response that can fully inhibit the infection in comparison to receiving BCG via skin which is not as effective.

2.9 New drugs and drug regimens to treat TB:

The new guidelines assessed by WHO in 2022 on latest drug regimens:

- 1) The strategy developed by WHO for new drugs authorization is treatment for six months with bedaquiline, pretomanid and linezolid (BPaL) followed by moxifloxacin (BPaLM) which is checked out in the clinical trial TB-PRACTECAL (Chang et al., 2018). From this BPaLM is said to be used for MDR-TB patients periodically without previous exposure of above drugs (World Health Organization 2022).
- 2) The 6-month regimens based on the BPaL has also been evaluated and finally the amount of linezolid doses have been identified (World Health Organization 2022).
- 3) The other modification in all-oral short regimens (6 & 9 months) is in continuous clinical trials. The previous stresses shown by WHO in 2020 related to patients of MDR/RR-TB & pre-XDR-TB, can now be relieved very much by this programmatic data study on new regimes.

2.10 The End TB Strategy:

WHO End TB Strategy 2016 - 2035

WHO's "Global strategy and targets for tuberculosis prevention, care and control after 2015" is taken up by World Health Assembly in 2014 (https://tbfacts.org/.). As Tuberculosis is a global epidemic, this 20 years planning and strategies were aimed to end this epidemic and called the End TB Strategy. This is defined as the occurrence rate of less than 10 people per 100,000 population per year. This incidence rate is the number of new active TB cases at a particular time in a population.

The major targets of this END TB Strategy are:

- Reducing the deaths by almost 95%, caused due to TB.
- To halt the new active TB cases by 90% between these 20 years.
- To make sure that because of this deadly disease no family feels burdened with unfortunate expenses.

2.11 Pathogenesis of TB:

The TB cycle starts with the dispersion of aerosols of Mtb. The risk of transmission arises when a dose of one bacilli spread throughout the air. When the bacilli enters the patient's lungs alveolar macrophages, phagocytosis starts. After this formation of granuloma started by the monocytes in response by immune system to fight off the disease. This is the hallmark feature of TB. Mtb secured prolonged survival within the alveolar macrophages. APCs (Antigen Presenting Cells), macrophages and dendritic cells involved in phagocytosis of pathogen in the lungs, fails to eradicate the infection. Among the most effective APCs dendritic cells play a crucial role during the initial stage of TB infection (Tascon et al., 2000). There are different Mtb components which are recognized and internalized by various host receptors like Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD), NOD like receptors (NLRs) and C-type lectins receptors (CLR). Toll-like receptors are TLR1, TLR2, TLR4, TLR7, TLR8 and TLR9, Nod-like receptors are NOD1, NOD2, NLRP3 and NLRC4 whereas C-type lectin receptors include mannose receptor (MR-CD207), the dendritic cell-specific intracellular adhesion molecule grabbing non-integrin (DC-SIGN), Mincle, Dectin-1 and Dectin-2, Dectin-3, CL-LK and DCIR. However, some are potential receptors such as complement receptors, scavenger receptors (SR), surfactant protein A receptor (Sp-A) and cholesterol receptors. Sp-A is a glycoprotein located at alveolar surfaces and it helps in enhancing the binding and uptake of Mtb pathogen by upregulating MR activity. On the bacterial cell wall, interaction of MR with Mtb is halted by blockage of manosyl oligosaccharide residues (Jo et al., 2008). Then inhibition of phagocytosis of Mtb started by surfactant protein-D. These host receptors identify micro bacterial cell wall mannose-capped Lipoarabinomannan (Man-LAM), etc. Phagosome maturation and cytokine signalling is also affected by the up taking of Mtb through different receptors (Philips and Ernst 2012).

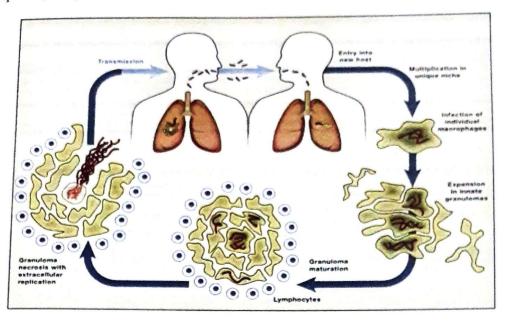


Figure RL4: Pathogenesis pathway of Mtb in Humans (Cambier et al., 2014).

When Mtb and other intracellular pathogens enter into the host macrophages, phagosomes are inhibited at the early stages. The phagosome-lysosome fusion is a normal maturation cycle by which bacteria faces harsh environment like low pH, toxic peptides and reactive oxygen intermediates (ROI) and lysosomal enzymes. Major elements in antimicrobial activity are reactive nitrogen intermediates (RNIs) produced by activated mouse macrophages. Usually the successful fusion of lysosome and phagosome results in the eradication of pathogen but in case of Mtb, phagosome consisting Mtb prevents fusion with lysosome, resulting in the persistence of Mtb within the host.

2.12 Introduction to MarR:

Evolution has led to the emergence of super powerful sensing and survival systems in all organisms that aids to their existence. Excellent adaptive capacity to the diverse set of stresses being faced during infection results in the successful intracellular infection of Mtb. These stresses are oxidative stress agents, organic solvents, lipophilic compounds, antibiotics etc. The proteins of multiple antibiotic resistance regulator (MarR) family respond to wide variety of stresses by modulating the expression of proteins. MarR homologs are also found to be involved in the regulation of virulence genes of pathogenic bacteria because of their ability to

respond to the sudden environmental changes. The MarR family proteins have a direct link in regulation of genetic loci conferring resistance to multiple antibiotics and pathogenesis (Perera & Grove 2010; Grove 2013). During environmental stress a signal molecule comes and binds to the DNA binding site of MarR protein, disturb the binding and switch on the regulation of the respective operon, diagrammatically represented in Figure RL5.

Therefore, the critical role of MarR family transcriptional regulators in the survival of Mtb by modulating gene expression under various redox stress conditions is crucial to study.

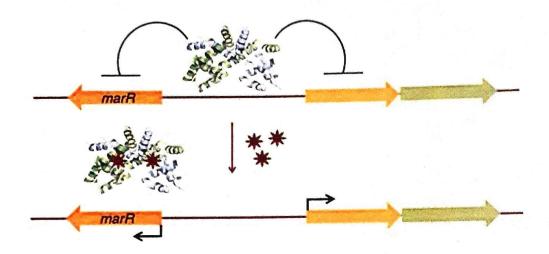


Figure RL5: A typical organization of genetic loci encoding MarR proteins (Zheng et al., 2019).

2.12.1 MarR Family Proteins of Mtb

MarR proteins came to existence in E. coli K12 and described as a transcription factor that controls the expression of marRAB operon and provides resistance to bacteria against antibiotics, phenolic compounds, organic solvents and other type of stresses (Alekshun and Levy 1999, 2007). MarR family factors are present extensively among other pathogenic bacteria of humans. Some of them are Salmonella typhimurium, Pseudomonas aeruginosa, Enterobacteriaceae and Bacillus subtillis (Gong et al., 2019). There is a lot of information available about the physiological functions of MarR protein in bacteria other than Mycobacterium. For example: there are 8 MarR homologs found in Mtb, out of which one is shown below:

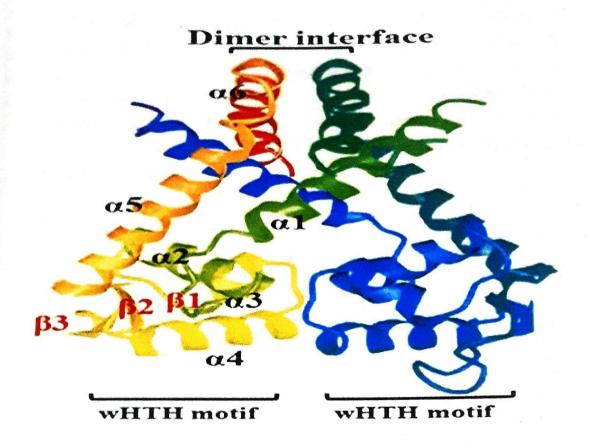


FIGURE RL6: Crystal structure of Rv0880 MarR family protein is resolved at 2.0A. The subunit of the dimer consists of six α-helices and three β -sheets. Each subunit contains winged helix-turn-helix (wHTH) motif as DNA-binding site (Gong *et al.*, 2019).

Just like these bacteria, the existence of MarR system also confirmed in Mycobacterium (McDermott *et al*, 1998) and identified that it is involved in drug resistance and intracellular survival of Mtb (Xu *et al.*, 2019). There are only eight MarR homologs identified till date, namely Rv0042c, Rv0880, Rv2011c, Rv1049, Rv2327, Rv0737, Rv2887 and Rv1404. Three out of theses eight proteins, Rv0880, Rv1049, Rv2887, have been characterised so far. Rv0042c one of the five uncharacterized MarR proteins.

2.12.2 Rv0042c

Rv0042c is a one of the functionally uncharacterised protein of MarR family in Mycobacterium. It is supposed to act as a transcription regulator in Mtb and found to be overexpressed in the granuloma site of human lung of a TB patient and associated with drug resistance. Rv0042c is 627bp/208 amino acids (~22.0 kDa) and is found in inner membrane in

cytosol of Mtb. Rv0042c is downregulated by a PhoPC which is a DNA binding domain of response regulator (Wang et al., 2007). Rv0042c is annotated to have helix-turn-helix DNA binding domain upon comparing its amino acid sequence with MarR protein of E. coli (Hao et al., 2014). There is a probability that Rv0042c may also play a role in binding to promoters of different genes via helix turn helix motif and regulate the expression under various stress conditions. So, there is a high probability that Rv0042c may have many functions which are connected with each other.

Materials

And

Methods

3: Materials & Methods

3.1 Chemicals and Media

Molecular biology grade chemicals including EDTA (0.5M), SDS, Glycerol, Acrylamide, BSA, Sodium azide, Bradford reagent, APS, TEMED, PMSF, Sodium Orthovanadate, Benzamidine, Tween-20, Bromophenol Blue, Etbr, βME, DTT, CaCl₂ and Agarose were purchased from Sigma-Aldrich, India. LB media components (NaCl, Casein enzyme hydrolysate, Yeast extract and Bacteriological agar), Skim milk powder, Tris (2M, pH=7.4), NaH₂PO₄, Na₂HPO₄, were purchased from HiMedia, India. For protein purification, Ni-NTA Agarose resin was procured from Qiagen. Snake skin Dialysis membrane, Coomassie Brilliant Blue R-250, Methanol, Glacial acetic acid, Isopropanol, Tris and Glycine was also obtained from Thermoscientific. Protein concentrators 10.0 MWCO were obtained from Merck. BL21RosettapLyS were obtained from Qiagen whereas pET28a plasmid expression vectors were purchased from Novagen. Nitrocellulose membrane used for western transfer was procured from GE Healthcare. Kanamycin and Chloramphenicol were purchased from Gold Bio and Sigma-Aldrich, respectively.

3.2 Competent cells preparation using CaCl₂ method

Primary bacteria was obtained by inoculating single colony in 10 ml culture bottle containing autoclaved LB broth followed by incubation at 37°C, 200 rpm for 12 h. The secondary culture was obtained by inoculating 1% primary culture in 25 ml LB broth. The secondary culture was incubated at 37°C, 200 rpm till 0.4 OD600 and then the culture was incubated on ice for 10 min. After 10 min, the cells were pelleted at 4,000 rpm for 5 min at 4°C in swinging bucket rotor. The pellet was resuspended by adding 10 ml pre-chilled Solution-A (0.1 M CaCl₂) and incubated on ice for 1 h followed by centrifugation of cells at 4,000 rpm for 3 min at 4°C in swinging bucket rotor. Supernatant was discarded and pellet was resuspended in 2 ml of pre-chilled Solution-B (0.1 M CaCl₂ + 15% Glycerol). Then, 100µl of prepared competent cells were aliquoted in pre-chilled and labelled MCTs and stored at -80°C until use.

3.3 Transformation of bacterial competent cells by heat shock method

A single aliquot of competent cells prepared as mentioned above was thawed on ice-water and then, 50 ng of plasmid DNA was added to the competent cells and incubated on ice-water for 15 min. Heat shock was given at 42°C for 90 sec and immediately plunged back into ice for 5 min. After 5 min, 1ml of autoclaved LB media was added to the cells and incubated at 37°C.

200 rpm for 1 h. The culture was plated on LB agar petri-dishes along with specific antibiotics for selection of transformed bacteria and incubated at 37°C for 12 h.

3.4 Plasmid isolation by Alkaline Lysis method

Primary culture was given in 10 ml LB culture bottle by inoculating single colony followed by incubation at 37°C, 200 rpm for 12 h. The 1.5 ml of culture was transferred in sterile MCT and centrifuged at 12,000 rpm, 1 min at 4°C or at 7,000 rpm, 5 min at 4°C. Supernatant was discarded properly and 100 µl of an ice-cold Solution I was added. The pellet was resuspended by tapping and kept on ice for 5 min followed by addition of 200 µl freshly prepared Solution II (Lysis buffer) and was mixed gently by inverting the tube 2-3 times. The tube was kept on ice for 5 min followed by addition of 150 µl of ice-cold Solution III (Neutralization buffer), mixed gently, kept on ice for 5 min and centrifuged for at 12,000 rpm for 15 min, 4°C. Supernatant was transferred into a fresh MCT and equal volume of Phenol: Chloroform was added, mixed properly and followed by centrifugation at 12,000, 2 min, 4°C. The upper aqueous layer was transferred to a previously chilled fresh tube and equal volume of ice-cold isopropanol was added. Mixed properly and kept on ice for 5 min. Centrifuged at 12,000 rpm for 10 min at room temperature and supernatant was discarded. Pellet was air-dried at 37°C for 30 min and resuspended in 50 μl of Tris-EDTA buffer (pH 8.0) followed by RNase treatment at 37°C for 10 min. Finally the concentration of DNA was determined through Nano-drop by taking absorbance at the ratio of 260/280 nm.

3.5 DNA purification by Gel Extraction Method

Gel Extraction method is used to extract and purify DNA using QIAGEN Gel Extraction kit which has yellow coloured QG buffer and PE buffer (ethanol is added before use). At first, excise the DNA fragment from the agarose gel with a clean, sharp scalpel. Weigh the gel slice in MCT, before that tare the weight of an empty MCT. Add 3 volume of gel to 1 volume of gel weighed then incubate it at 60°C in a heater for 10 min until gel dissolved completely and vortexed for 2-3 times during incubation. Checked the mixture colour. Added 1 gel volume (equal to gel weight) of isopropanol to the sample and mixed. Placed a spin column in 2 ml collection tube. To bind DNA, applied the sample to spin column and centrifuge (1 min, 11,000 rpm) at RT, discarded the flow through. To wash, added 750 µl of buffer PE into spin column, centrifuge (1 min, 13,000 rpm), discarded the flow through and centrifuged an empty spin column (1 min, 13,000 rpm). Placed spin column into a clean 1.5 ml MCT then eluted DNA,

added 30 µl EB buffer (added according to amount of sample DNA) to the centre of the spin column. Stand still for 1-5 min then centrifuge for 1 min then purified DNA was eluted into MCT. The reading of purified DNA was taken in Nano Drop.

3.6 Protein purification for Rv0042c and its mutants

3.6.1 Over-expression of proteins:

Primary culture was given by inoculating a single colony of BL21Rossetta pLysS cells transformed with vector pET-28a (Rv0042c) for His-tagged Rv0042c proteins in 10 ml culture bottle followed by incubation at 37°C, 200 rpm for 12 h. From primary culture, 1 % inoculum was given for secondary culture in 600 ml LB media and incubated at 37°C, 200 rpm until OD600 reached 0.4 followed by cold shock treatment for 30 min, then kept the culture in 18°C at 200 rpm in shaker for 20 min. After this take out 1.0 ml uninduced sample, then, induced with 0.5 mM IPTG and incubated at 18°C for 18 h. After 18 h of induction, cells were harvested at 6,000 rpm for 10 min at 4°C. The supernatant was discarded and the obtained pellet was resuspended in 1X PBS (pH 7.4) with the help of vortex, centrifuged at 6,000 rpm for 15 min at 4°C discarded the supernatant and proceeded with pellet for protein purification.

3.6.2 Bioinformatics:

Rv0042c sequence alignment was done with other well studied MarR family proteins to find out the present identity. Alpha fold and pyMOL software were used for structural visualization of Rv0042c.

3.6.3 Purification of His tagged Rv0042c protein:

The induced pellet was resuspended in 10 ml lysis buffer containing 0.2 M Orthovanadate (30 μl) and 100 mM PMSF (300 μl) protease inhibitors. Then dissolved the pellet completely and volume was made up to 30 ml, after this added 5 μl DNase enzyme, 35 μl β-ME, 30 μl Triton-X 100 and kept it for 20 min. The resuspended pellet was then sonicated at 15 pulse "On" and 20 "Off" having amplitude 24 % till the pellet became translucent. After sonication, the lysed cells were centrifuged at 11,000 rpm for 30 min at 4°C. The supernatant was transferred to the fresh Falcon tube and loaded onto a Ni-NTA column pre-equilibrated with 10 mM Imidazole buffer. The supernatant was passed 5 times through the column. The column was washed with 30 ml of wash buffer comprising 500 mM NaCl, 20 mM Tris and 10 % glycerol and increasing concentrations of Imidazole buffers, i.e., 20 mM, 40 mM, 60 mM and 80 mM (pH 7.4). The

protein was eluted with 10 ml of 250 mM elution buffer and the elution fractions were collected. The eluted fractions containing desired protein as analysed by SDS-PAGE were pooled and dialyzed in dialysis buffer 500 mM NaCl, 10 % glycerol 20 mM Tris and 0.5 mM EDTA (pH 7.4) for overnight at 4°C on stirrer. Next day, the protein was concentrated using 10 MWCO protein concentrators. The concentrated protein was quantified using Bradford assay at 595 nm.

3.7 SDS PAGE

The purified elution fractions and samples from washings along with uninduced, induced supernatant, pellet and flow through were electrophoresed on 10% SDS-PAGE after adding 4X loading dye. Prior to loading of sample onto the gel, the samples were heated in a dry bath at 100°C for 10 min and centrifuged 13,000 rpm for 1 min at RT. The samples were analysed on discontinuous vertical SDS-polyacrylamide gel with 5% (w/v) stacking gel and 10% (w/v) resolving gel using 25 mA current. Protein molecular weight markers were run concurrently on the same gel for analysis. Gels were stained with Coomassie Brilliant Blue staining solution followed by destaining.

3.8 Bradford Assay for quantification of protein

Protein samples were prepared by diluting the concentrated protein samples 1:10 or 1:5 in water followed by addition 500 μl of Bradford Reagent. The sample was mixed properly. Respective blanks were prepared by replacing the protein sample with dialysis buffer. The absorbance of blue colour product was measured on a UV spectrophotometer at 595 nm. For standard curve, the standard BSA solution was prepared with concentration 0.1 μg/μl, 0.2 μg/μl, 0.3 μg/μl, 0.4 μg/μl and 0.5 μg/μl from BSA stock of concentration 2 mg/ml. To prepare the standard curve, absorbance value at 595 nm of each standard BSA solution was plotted against their concentration and a linear curve was obtained. The unknown concentration of protein was calculated by comparing their absorbance at 595nm with known BSA standards using standard curve and expressed in μg/μl.

3.9 Non-denaturing SDS Page

Rv0042c and its deletion mutants were treated with β -ME and DTT followed by boiling the samples at 100°C. Other set of samples without the treatment of β -ME or DTT were not boiled.

All these samples were subject to SDS-PAGE to check the oligomerization status of the proteins. Results were confirmed by western transfer.

3.10 Western blotting

The protein samples electrophoresed in SDS-PAGE gel was transferred onto a nitrocellulose membrane in a Trans-Blot apparatus. The membrane was stained with Ponceau S stain and washed with 1 % Acetic Acid until the stain was completely removed. The membrane was blocked in 5% skim milk for 40 min at RT. The blot was washed thrice with 1X PBST for 10 min each and incubated with particular dilution of primary antibody, i.e., Anti- His at 1:20,000 dilution for 2 h followed by 3 washings of 10 min each with 1X PBST and incubated at RT with enzyme-conjugated secondary antibody, i.e., Goat Anti-mouse at 1:15,000 dilution for 1.0 h. After washing thrice with 1X PBST, appropriate enzyme substrate solution was added. Membrane was exposed to the X-ray film for various time points and developed in a dark room.

3.11 Gel Filtration Chromatography- FPLC (Fast Protein Liquid Chromatography)

AKTA Purification: This is a technique often used to analyse or purify mixtures of proteins in which the molecules are separated according to difference in sizes when pass through a column packed with the chromatographic medium (gel) formed from polymers. Pores in gel matrix are filled by liquid phase and are comparable in size to the molecules we wish to separate. Small molecules can diffuse into the gel from surrounding solution, relatively large molecules will be prevented by their size from diffusing into the gel. As large molecules are unable to diffuse and confined to outside solution, they can come down to the column very fast as an eluent. In FPLC the mobile phase is a liquid and the stationary phase is often a resin. Fast protein liquid chromatography systems generally consist of a pump, a UV detector, a conductivity meter, and a fraction collector and operate at pressures of ~3,500 psi (24 MPa). Samples can either be loaded manually by injection into a sample loop or automatically using a sample pump. Some fast protein liquid chromatography systems have multi-wavelength detectors for monitoring sample elution at several wavelengths.



4. RESULT

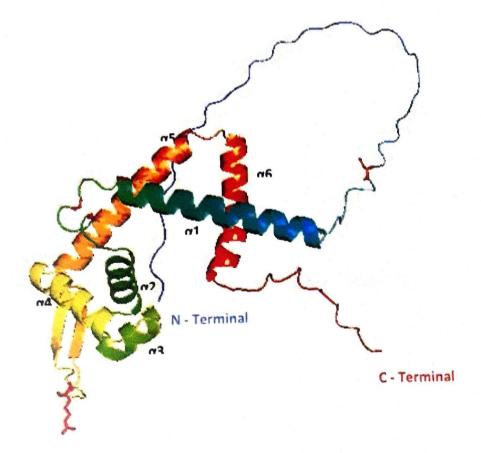
4.1 Introduction

Rv0042c is an important MarR family protein which is a possible transcription regulator existing in oligomers under native conditions in Mtb. Therefore, it is crucial to study the oligomeric nature of this protein. To unravel the oligomeric status, Rv0042c was cloned from genomic DNA of Mtb by the senior in the lab and its purification was standardized. Later on analysing its structure through alpha fold model, it was observed that this particular protein contains long unorganised loops at its 'N' and C' terminal. To study the effect of these loops on the proper folding and structural stability, 'N' and C' terminal deletion mutants were also generated by a senior in the lab and their oligomeric behaviour have been compared with the wild type by me.

4.2 Analysis of predicted structure of Rv0042c on Alpha Fold

Due to unavailability of crystal structure of Rv0042c, Alpha Fold software freely available at Google was used to predict the structure (https://alphafold.ebi.ac.uk/search/text/rv0042c). Looping regions were identified and deleted using sequence specific primers.

A.



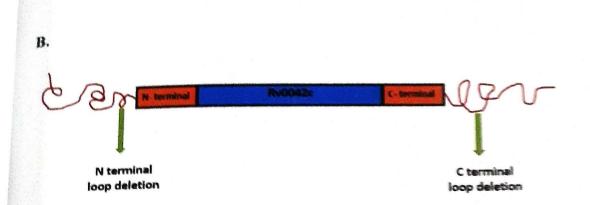


Figure R1: A. Predicted Structure of Rv0042c at α fold. Comprising 6 α-helices and 3 β-sheets, a typical winged helix-turn-helix structure of MarR family proteins. B. Diagrammatic representation of deletion of long loops from N and C terminal domains of wildtype Rv0042c.

4.3 Preparation of Rosetta pLysS competent cells and Transformation-

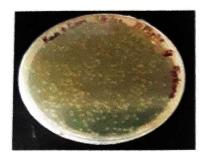
BL21 Rosetta pLysS competent cells were prepared using CaCl₂ which aids and binding of plasmid DNA to lipopolysaccharide present in cell wall. Positively charged calcium ions attract both negatively charged DNA backbone and the negatively charged group in the LPS inner core. During transformation of these cells by heat shock method, a sudden increase in temperature created pores in the plasma membrane of bacteria which allowed plasmid DNA to enter the bacterial cell.



A.



B.

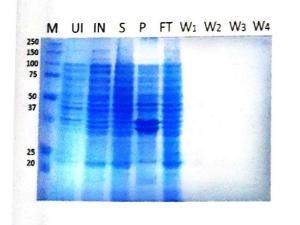


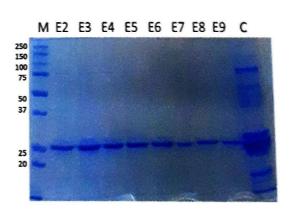
C.

gure R2: Transformation of BL21Rosetta pLysS cells- A. Competent BL21Rosetta pLysS pression cells transformed with vector pET-28c containing Rv0042c at T7 promoter. (A) Agar ite (Kan + Cam) showing single colonies for wild type transformants B. Agar plate (Kan + Cam) owing single colonies for N terminal deletion mutant. C. Agar plate (Kan + Cam) showing single lonies for C-terminal deletion mutant.

4.4 Purification of Rv0042c Wild Type Protein with N-Deletion & G-Deletion Mutants

Rv0042c wildtype protein was purified using affinity chromatography. The various fractions of purification process were electrophoresed on SDS-PAGE, purified band appeared at 25 kDa (Fig. R3). The N and C terminal deletion mutants were purified by the same protocol. Both mutants were eluted around 50 kDa (Fig. R3 & R4), indicating that it is present as a dimer.

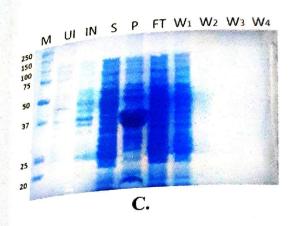




A.

B.

matography. Panel A. Lane M = marker; UI = Uninduced sample; IN = induced samples; S = matant; P = pellet; FT = Flow through; W1 = 20 mM washing; W2 = 40 mM washing; W3 = 60 washing; W4 = 80 mM washing. Panel B comprise the elution fractions of purified Rv0042c from E9 followed by C = concentrated protein.



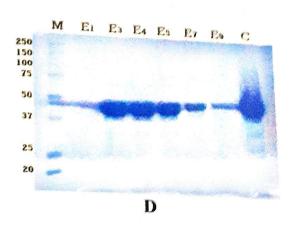
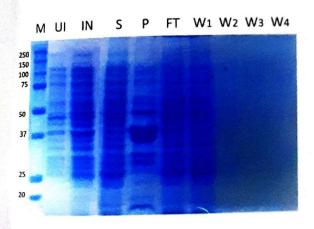
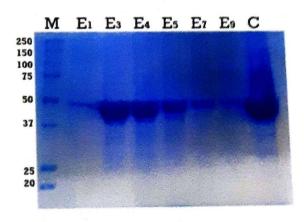


Figure R4: Showing the purification of N-terminal deletion mutant of Rv0042c protein by using affinity chromatography. Panel C. Lane M = marker; UI = Uninduced sample; IN = induced samples; S = supernatant; P = pellet; FT = Flow through; W1 = 20 mM washing; lane W2 = 40 mM washing; W3 = 60 mM washing; W4 = 80 mM washing. Panel D comprise the elution fractions: E1, E3, E4, E5, E7, E9 followed by C = concentrated protein of purified N-deletion mutant of Rv0042c. protein.





E.

F.

Figure R5: Showing the purification of G-terminal deletion mutant of Rv0042c protein by using affinity chromatography. Panel E. Lane M = marker; UI = Uninduced sample; IN = induced samples; S = supernatant; P = pellet; FT = Flow through; W1 = 20 mM washing; lane W2 = 40 mM washing; W3 = 60 mM washing; W4 = 80 mM washing of C-terminal deletion mutant of Rv0042c Protein. Panel F comprise the elution fractions: E1, E3, E4, E5, E7, E9 followed by C = concentrated protein of purified G-deletion mutant of Rv0042c protein.

4.5 Oligomerization of Rv0042c and its mutants:

Purified Rv0042c and its deletion mutants were electrophoresed under denaturing and non-denaturing conditions. One sample from each protein was treated with 10 mM β-Mercaptoethanol (β-ME) and followed by boing at 100°C for 5 min to ensure proper denaturation of protein. The protein sample not treated with β-ME and no boiling was kept as control. It was observed that under non-denaturing conditions, wildtype Rv0042c exist as a dimer (50 kDa) which was reduced to monomer (25kDa) when treated with β-ME and upon boiling (Fig. R7. A) In case of 'N' and 'C' terminal mutants, tetrameric form was observed under non-denaturing conditions and stable dimers were found at denaturing conditions. Same results were confirmed using DTT at 10 mM as a reducing agent (Fig. R7. Panel B).

To ensure the proper reducing environment deletion mutants were also titrated against higher reducing environment so that the dimer can get reduce to monomer as in case of wildtype Rv0042c. However, no effect was observed on these stable dimers in case of 'N' and 'C' terminal deletion mutants. These result highlighted the importance of loops in folding of the protein structure.

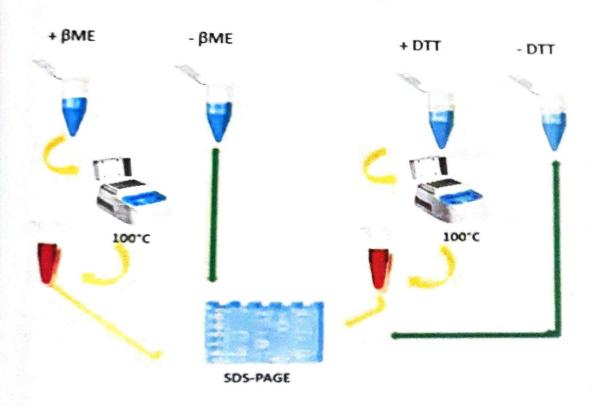


Figure R6: Flowchart of the experiment.

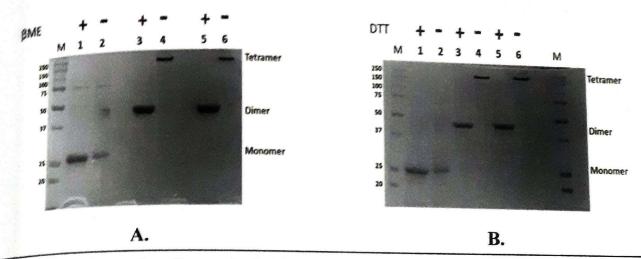


Figure R7: Shows the oligomeric forms of Rv0042c wildtype and 'N' and C' terminal deletion mutants.

- A. Wildtype and mutants treatment with β ME as a reducing agent, lane M = Marker; 1 = $+\beta$ -ME treated and boiled wildtype Rv0042c, 2 = $-\beta$ -ME, unboiled wildtype Rv0042c; 3 & 4 shows β -ME treated and Non β -ME treated proteins for N terminal deletion mutant and 4 & 5 = β -ME treated and Non β -ME treated proteins for N terminal deletion mutant non-reduced C.
- **B.** Similar treatment was given with DTT as a reducing agent: Lane 1 & 2 shows Rv0042c Treated and Non- treated samples, 3, 4, 5 & 6 shows same for 'N' and 'C' terminal deletion mutants respectively.

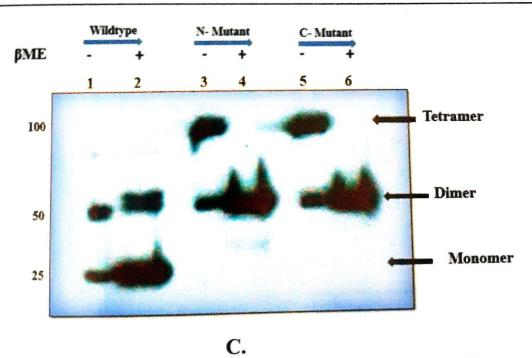


Figure R8: Shows the western Blot analysis of the previous experiment. Confirmation of the oligomeric forms of Rv0042c and its deletion mutants at 'C' and 'N' terminal through western blotting with α -His antibody.

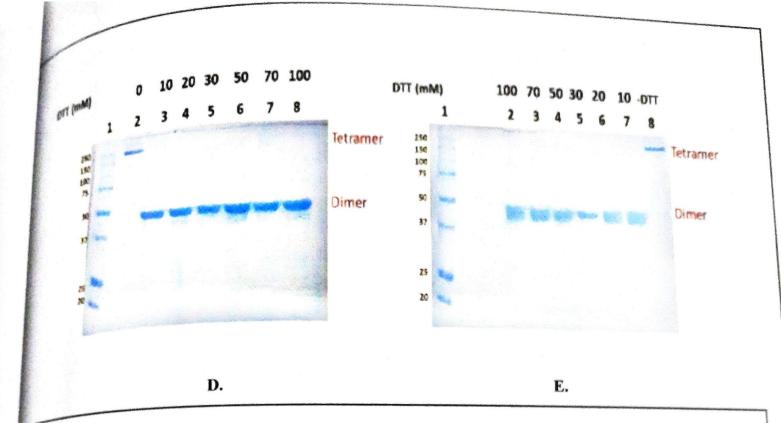


Figure R9: Shows the effect of Increasing concentration of DTT on the oligomeric behaviour of 'N' and 'C' terminal deletion mutants of Rv0042c. Panel C and D shows the effect of increasing concentration of DTT as a reducing agent from 10 mM to 100 mM in comparison to 0 mM DTT in lane 2 in case of N-terminal deletion mutant and in lane 8 in case of C-terminal deletion mutant respectively.



5. Discussion

pespite the availability of highly effective drugs, world is facing the problem of treatment of TB even in 21st century. The problem of emergence of drug resistance further aggravates this crisis. MarR family protein are promising candidates for meeting out the drug resistance as they have the direct link in regulating the efflux pump and other stress responsive genes to sustain the survival of the pathogen. In case of Mtb elaborative study of this family of protein is still lacking.

Rv0042c selected in this study is indicated to be involved in drug resistance according to the literature. In the current study, it was found that like other MarR proteins wildtype Rv0042c exist as a Dimer in its native conditions. Dimers appeared to be the functional form of Rv0042c as it binds its specific DNA sequence. Under reduced conditions, it exist as monomer. The long loops of this proteins have a greater role in the stabilization and perhaps provide flexibility to the protein to adapt according to the surrounding environment. Deletion of loops from N and C terminal of wild type Rv0042c provided more rigidity to the dimers and tetramers as they are not converting to monomers even at higher concentration of reductive agents.

Rv0042c can be a global regulator of Mtb to sustain its survival under varying stress. Further, studies are needed to look into its regulation and functionality *in vivo* as it is important to reveal the intriguing interconnected regulatory networks of Mtb for better understanding of its virulence and pathogenesis.

Conclusion

6. Conclusion:

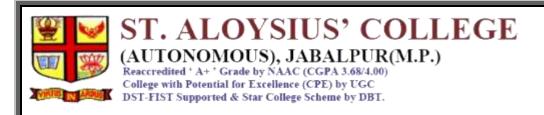
Rv0042c is an important member of MarR family of proteins. For any protein study its structural features are crucial in reference to its function. In this study, the oligomeric behavior of Rv0042c under oxidative and reductive conditions have been analyzed. Interestingly, Rv0042c forms dimers in its native form and its N and C terminal long loops are crucial for its oligomeric behavior and stability. After loop deletions, Rv0042c forms rigid dimers and tetramers even under harsh reductive conditions which indicates the involvement of loops in sensing its outside environment and change its oligomeric structure accordingly.

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SAMPLE PROJECT REPORTS 2021-22 FACULTY OF ARTS

Page 2



Field Project Work Report

प्रोजेक्ट (परियोजना) कार्य

Consumer Right

(Title of Field Project Work)

For the partial fulfillment of B.A./ B.Sc./ B. Com./ BBA/ BCA Degree बी.ए. / बी.एस.सी. / बी.कॉम. / बी.बी.ए. / बी. सी.ए. / की डिग्री की आशिक प्रतिपूर्ति हेतू

Session: 2021-22



Oct of Political Science Con

St. Aloysius' College (Autonomous)

Reaccredited 'A+' by NAAC (CGPA 3.68/4.00)
College with Potential for Excellence by UGC
DST-FIST Supported & Star College Scheme by DBT
Jabalpur, Madhya Pradesh, India

Field Project Work Report परियोजना कार्य रिपोर्ट

Constant Right.
(Title of Field Project Work)

For the partial fulfillment of B.A./ B.Sc./ B. Com./ BBA/ BCA Degree बी.ए./बी.एस.सी./बी.कॉम./बी.बी.ए./ की डिग्री की आंशिक प्रतिपूर्ति हेतु

Session: .. 2021 - 22.....

Name of student/ Students (छात्र का नाम)

Bo A 182 year

Class (कक्षा)

Roll No (अनुक्रमांक)

St. Aloystus College जनिक्या Name of the organization, where the work was completed (संस्था का नाम जहाँ कार्य पूर्ण किया गया)

Dr. Vishwas Patel.

Name of Supervisor (पर्यवेक्षक का नाम)



St. Aloysius' Conege (Auto

St. Aloysius College

(Autonomous)

Reaccredited 'A+' by NAAC (CGPA – 3.68/4.00)
College with Potential for Excellence by UGC,
DST FIST Supported & Star College Scheme by DBT
Jabalpur (M.P.) India

Declaration of Student's Original Work विद्यार्थी की मौलिकता का घोषणा पत्र

this Field Project report is based on the original published and unpublished material has been us declare that the submitted report has not been sulcourse in the past / present.	work done by me, in which ed after due approval. I also		
4	एतद द्वारा		
घोषणा करती / करता हूँ कि यह परियोजना रिपोर्ट	मेरे द्वारा किये गए मूल कार्य पर		
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उपरान्त किया गया है मैं यह भी घोषणा करती / करता हूँ कि प्रस्तुत रिपोर्ट किसी अन्य			
डिग्री / पाठ्यक्रम हेतु पूर्व / वर्तमान में प्रस्तुत नहीं किया गया है			
Name of student/ Students (छात्र / छात्रा का नाम)	: Price WUMOT Bais		
Class (कक्षा)	: B.A Jot year		
Roll Number (अनुक्रमांक)	:		
Signature with date (हस्ताक्षर दिनांक सहित)	:		

Acknowledgement

Price Kuman Bals of class B.A. I. year, a Aloysius' College (Autonomous), Jabalpur would like to express my ratitude towards our Principal Rev. Dr. G. Vazhan Arasu for giving me this reat opportunity to do this Field Project Work.
Department of Political Science for his / her constant guidance, advice, encouragement & every possible help in the completion of this work.
The field project opportunity I had with [Name of the Company] was a great
chance for learning and professional development. I express my deepest gratitude and special thanks to the [name of authorized person]who
in spite of being extraordinarily busy with her/his duties, took time to listen to my queries, to guide me on the correct path and allowed me to carry out my field project at their esteemed organization.
am extremely grateful to all my teachers and friends who helped me in the successful completion of this field project work.
With great pleasure, I would also like to extend my sincere gratitude and thanks to my parents for their support. I am grateful to everyone who supported me or guided me directly or indirectly.
Signature
Name of the student :

INTRODUCTION

Consumer's Rights In India

The Definition of Consumer Right is The Right to have information about the quality, potency, quantity, purity, price and standard of goods or Services; its it may be the case, but the consumer is to be protected against my unfair practices of trade. It is very Essential for the consumers to Know these Rights

However there are strong and clear laws in India to defend consumer Rights, the Actual plight of Consumers of India can be declared as completely Dismal, out of the various laws that have been Enforced to protect the consumer eights in India the most important is the consumor prestection Act 1986, According to this law Everybody orduling individuals, a form, a hindu undivided family and a company, have the night to exceeded their Consumer elights for the purchase of the yours

and services made by them. It is significant that, as consumer, one knows the

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	Field Project Working Notehook		
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Topic	Date	N	
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	Il as about the courts and follow the infringment of one's		
The state of the s	the Consumer Rights in India	1	
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* The Right to be po	sotected ferom all kind of hazardous	1	
Maria Caracteria de Caracteria	elly informed about the performance		
and quality of all			
* The Right to free	charce of Goods & Services.	M	
* The Right to be he	eard in all Decision-Making		
processes helated	to Consumer interests.		
* The Right to Seek	Redressal, whenever consumer		
rights have been i	infringed		
* The Right to Compl	ete consumer education.		
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Teacher's Signature

Right To Information

The Right to Information is defined as the Right to be informed about the quality quantity potency, purity and standard of police? of goods & services, as the case may be so as to protect the consumer against unfair trade practices in the Consumer protection Act 1986, In the market place of India, Consumers got Information By Tuo ways namely advertising and word of mouth however these Sources are Considered to be unreliable but still this would of mouth is quite Common here Because of this, the Indian consumers houdly have precise & complete information for adressing the true, value, Safety, Suitability, reliability of any product. usually the hidden costs can be found, lack of Suitability, ouality problems and safety hazards only after the purchase of the product. There is another Right claimed by the Indian government on paper. This Right nust ideally make seen that all consumable product have been labelled in a Standard mannes Containing the cost, quantity, the ingredients and Instructions given to use the product safely Leader Designer Sheets Teacher's Signature

		5	-	1	1
Topic .				Date	
	Donty	9- 01-			

Right To Choose,

The Definition of Right to Choose as post the Consumer protection Act is the Right to be assured, reduced possible, to have access to a variety of goods & services at Competitive prices.

for Regulating the Market place, There is just one factor required and i.e. Competition. The Existence of Cartels, oligopolies & Monopolies priore to be countriporductive to Consumerism. The Natural resources liques industry, Telecopomunications, Airlines etc.

All are being Controlled by a makin to some on other exent. Since the Indian Consumers Come from a socialist background, the Polerating of monopolistic market is found in their Blood.

Right To Consumer's Education

The Right of Every Indian citizen to have Education on Matters Regarding consumer protection as well as about health's right is regarded as the last right provided by the Consumers in the Country have informational purguans of materials which are Easily

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TopicDate
I I amalla thomato make purphasing
secisions which are believe than before.
Decisions with are percent
CON Y
* First Progress Report for Feild
Protect
Tho on
Introduction and scope of the Peroject work -
The state of the s
According to the Consumer Perotection Act 1986,
The Consumer right is referred to as the right
to be protected against marketing of goods & services
which are Hazardous and food processing, this
sight is spread across the Domain having a serious effect on the Health of the Consumor on their
will being viz Automobiles, Housing, Domestic Appliances
travel etc. when there is violation of the Right
then there occur medical malpractice in the country.
it is estimated every years that thousands or millions
of citizons of India are killed or Seriously injured
by Immoral practices by Doctors Hospitals, pharmacies,
and the automobile industry. Still the government
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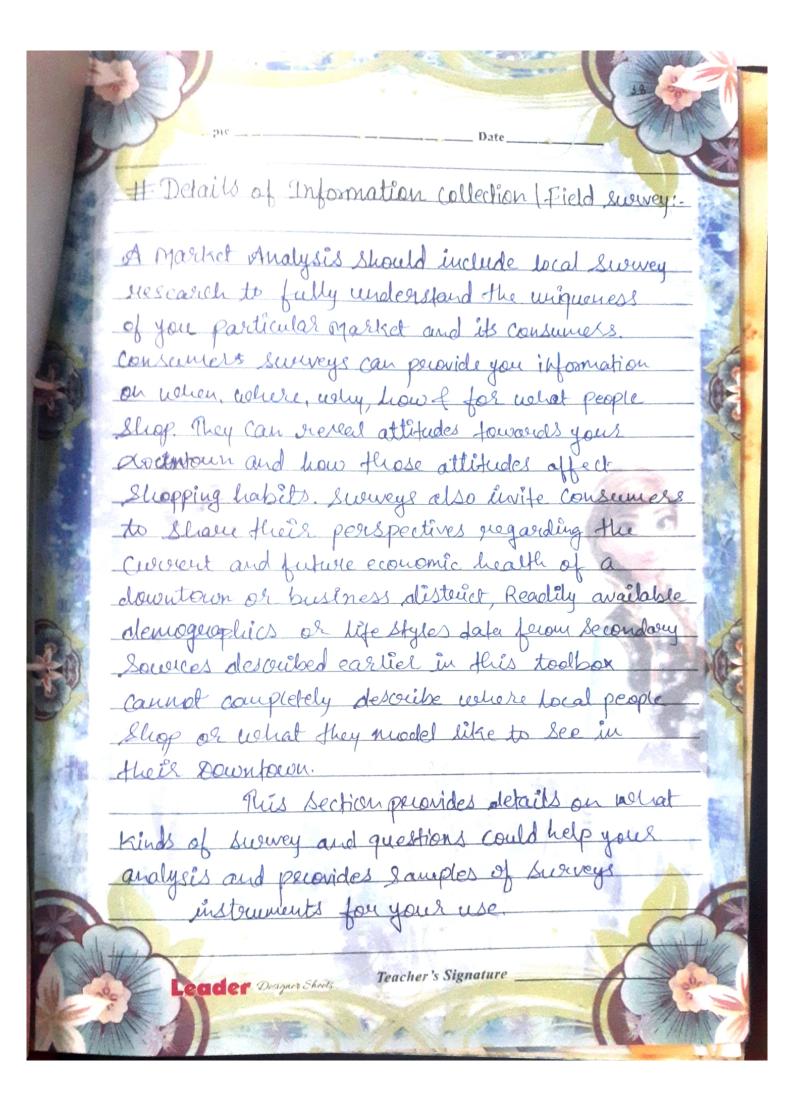
of India known for its callousness, does not Succeed in Acknowledging this fact or making a feeble effort for mainting statistics of the mishaps. The government of India needs to how world class product testing facilities to test duys, food, cars Or any other consumable product that can prove to be a menace to life. # Scheme structures of the Project work ~ The Right to Information is defined as the right. to be informed about the gustity, ourntity, potency, purity, standard and price of goods or services as the case may be so as to perotect the consumer orgainst unfair trade in the consumer protection Act 1986, in the market place of India consumers get information by tur ways namely advertising and word of north housier these sources are Considered to be undeliable but still this world of mouth is quite common here because of this the Indian consumers hardly have priecise & complete information for adressing the true value, safety suitability, reliability of any product usually the Leader Designer Sheets

TopicDate	
* Analysis: Study of collected inform	ation
* Interpretation: to find the result	from it.
* conclusion	
## Details of the concerned work place (where the work has been done	e institution
1. Sadar Bazaar	
2. city	
3. Georgklipur	- T
4. Madan Mahal	
5. civil line	
6. Chanta gliar	
1. Railway Station Etc.	
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Topic	Date
# Peroposed peroject Groats Sh	ould be short and
Smart ~	
The Right to seek hedressal	against unfail
trade practices or restriction	re trade peractices
de unscempulos exploitation	of consumers is
age to as the right of	to Hedressal acrosiding
to the consumer perotection	Act 1986.
The government of India hors	been hit more
Successful with negard to	His week a
Lours et district consum	18 del sul la
formens at district land.	Sloke Conserved Discola
Hedressal commission have b	2010 Misseuer Dispiete
The help of the consumer po	untockou All reas
Nese consumos agricuarios	11 10 10 10 10 1986.
These consumers generances	Hedressal agencies
Twelsdictions will all all	geographical
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The cases and He	28/28 20-1
answer Consumos Lou	11111 01 1 0 1
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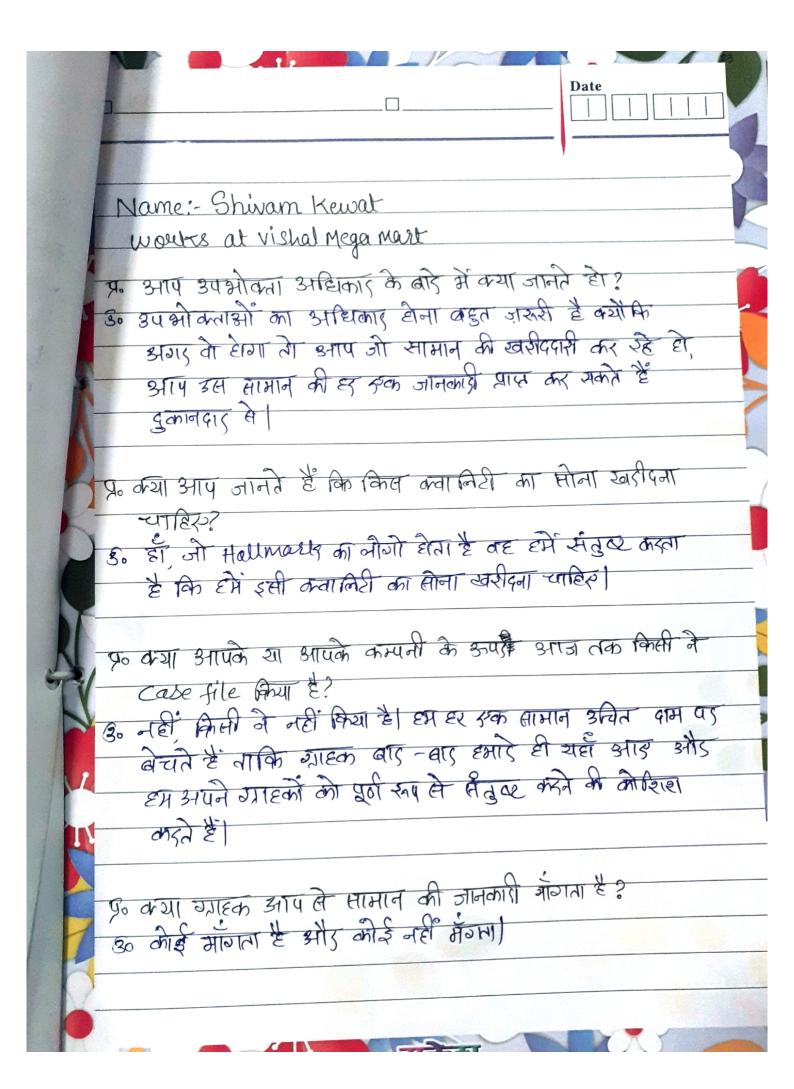
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K		- A
B	Date	
	## Second Progress Report of Project work-	
	# Peroject Methodology (work flow)	X
	consumers are the largest economic group in any country. They are the central point of all	4
	economic activities. The ultimate of aim of	
	ensure consumption of goods & & services to the	
	Satisfaction of the consumers. consumer awareness means being conscious of having	
	Laues. Hedress mechanism and the Consumer buy	
	get representation if there is any government of	
	Suggestion, and sight to Seek redress agains unfail trade practice or inscrupulous explointation	1
推	The conserver protection Act was exacted in 1986 to protect the conservers from unfair brade	
	plactice.	
		7
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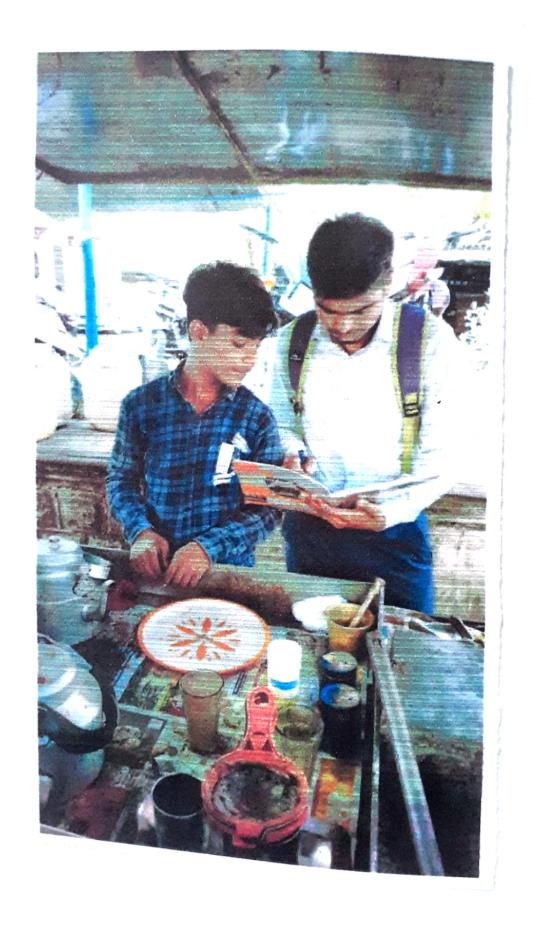
	pie Date	
E	Literature Review ~	
M	Though the first consumer movement began in	
置	England after wwII, a modern declaration	3
E	about consumer's eights was first made by	
	the former US. President John F. Kennedy	
	befollethe US congress on March 15, 1962 voluere	
	four basic consumer rughts were recognised	
	the night to safety & the night to be	
	informed and the right to choose. This is	6
	acted as the springboard for research in this	
	area. After that a number of research	-
	Hesearch Studies have been undertaken on	
	conserver right.	-16
		- 7
		15
K		A
	Leader Designer Sheets Teacher's Signature	Do les
War.		
1		11 1





Date
Name:- Bhavilt Agrawal Men's Selection
8. Do you examine the expiry date of products?
A-yes.
a. Do you know about consumed formen?
4- NO.
Q. Do you sell your product at MRP)
A- yes, and sometime below MRP to attract
a what so you would do if other shopkeeper is explaining a consumer?
A-I can case a file at consumer forum on him.
TICTAL

DateDate
NAME: NIKHIL JAIN
9 to 11 Gustar Namkeen
8. Do you examine expiry date of a product when you
A- yes.
4- 400.
8. Do you sell your products at MRP?
A- Yes.
& what will you do it any consumor cases a files
A- Don't know. 1'11 sealch in internet
TOM TOWN. I TOWN
Q. Do you give the Bill of the Consumer?
A-yes, Always.
NAME: Abhisha vince PIVAT
Student
9. what Do you know about consumer's rights?
A- Every consumer should know their nights
to be safé praudent.
Leader Designer Sheets Teacher's Signature



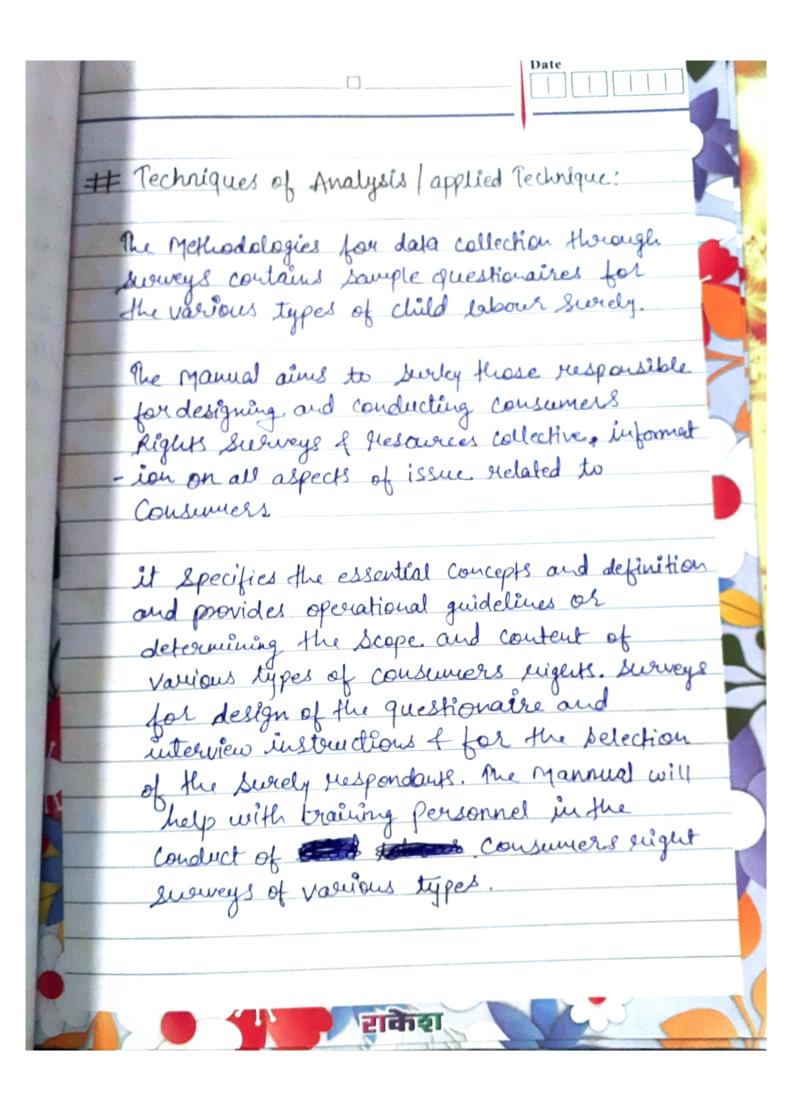
Date	
Name:- Kocishnapal singh Steedent	
प्र Consumer Perofection Act क्यों नागू कियां गर्या शा भ	<u>प्रतिथ</u>
3. ये जी consumes perotection Act है र इसे २५ दिसंबद् 1986 की लागू कियाँ गर्या था किंग्स की सरकाद द्वारा जाग्य	(किया)
गया था और उस समय के प्रधान मेत्री याजीव जाँखी है। इस रमिक् कार्य किया गया था क्यों कि उपभीवनाउनी की अत्वत तरीके से सामान बेचने और सरी जान कारी न देने	न और
उप भोक्ता का interest बचाने के लिए लागू किया ज	ायां था
त्रिकार का हनन कर यहां है तो?	ৰূম
उठ उनगड़ में रक्त दुकान में गया और मुझे कोई समान ले हे ड्रोड़ मैंन रुक सामान जुन लिया पड़ वह दुकान पड़	
मुझे ज्बार्यसी वाह न्या कहरें है कि वह इस सामान ले जो, तो यह मेडे युनने का अधिकाह का ह	मर्न ।
To National Consumer's Rights day ond HATELT SIT	श हुडे
so 24 Rédas	
राकेश -	

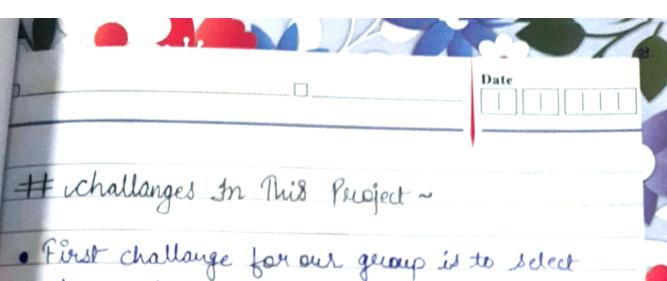
Har Third Progress Report of Project work # Details of the work Completed ~ Its like a task for me to visit lots of places to find peoples to gather the Information related consumers right. This peroject has made all of us a lot to l'earn from it. how we should conduct survey, how to interact with unknown peoples and how to express yourself. These kinds of project would help a lot in the future. we have also passed many slifficulties to complete this project. After visiting a lot of places with my guoup members we started searching for those people to whom we could ask the questions, but we could only find 1-3 posson at a time place or a day.

As of now we have gone thorough 8-11 Sweezes conducted at different per places. The main peoblem was to search for the people volio could answer to us. we went to many shops like real stall, paan shop Samosa Shop where labour gakurs daily color we asked some from to answer our question they refused to answer because of their manaveress about consumers sights & consumers courts. To falk with riterate peoples, firstly we have to convince them that we would not page your much of time So far we have completed most of our peroject work like sutroduction, P1, P2 etc it would take Some more days to complete flies peroject. This is how I complete my Side of works in this project. we learned a lot of things forom this project surley.

hazardous preodects, black marketing and so

on & so forth.





- · First challange for our geroup is to select places where we can get more people to interact with.
- Second Challarge was to prepare the questionaire in this we faced many challarges like what kind of question we have to designe, which kind of rocablury we can use, how many question we can ask, how to average question in series, some language is sue.
- · Nierd challange was, how to communicate with those peoples, how ask those question, how to talk to them, through which technique we could get more cooperation from them and many more challanges.
 - of those preoples were not able to answer our questions, Some people doesn't have time to answer the question of many of them have not cooperated with us.

Date

Field Project Final Report

Consumer Report Rights is a Subject flat has been Settled in most offer contries, but there are still many people volvo didn't know about the topic every is one entitled to the same nights no matter what country they live in and should be informed of their right one way this can be done is by specepling awareness on consumer orights through so vial Media.

consumed Rights are a very important topic in India. They have been grounted to the people of this country and are meant to the Indian constitution and are meant to provide protection against exploitative trade practices. Many people in India feel that they no longer need consumer Rights because they have less many and there will be less preducts available to them in the future as well as with more competition from outside companies.

India is a country with a population of over 1 billion people, within this lavge population, frede are a number of people will have not yet been informed about the nights given to flient by the govornment. These highes are Marry outlined by the consumer protection and allow for consumer to sue if they were whorsed by a company on hetailer. Anagine snegure you buy a shirt, and it falls apart after one wash. This has happened to so many people in India, especially since companies are taking advantage of the market. The majority of Consumers feel that their nights one not being Mespected and that they are not getting collab they paid for redule the government is only trying to help the companies make more profit. we conducted the survey based on a questionair we prepared. Some of the questionaire and peoples newark are as follows:

Consumer

Rights

