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# CASE STUDY ON ASSESSING STUDYING PATTERNS OF AERONAUTICAL ENGINEERING UNDERGRADUATES OF GENERAL SIR JOHN KOTELAWALA DEFENCE UNIVERSITY IN SRI LANKA

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## *ABSTRACT*

Study patterns consider as one of the prominent factors in academic achievements. These studying patterns can be evaluated under several parameters such as regular study, study space, efficient in reading, listening to lectures, active participation for the lessons, doing homework, efficient writing of notes, preparation for exams and attending for exams. In this research, the sample consisted of all the undergraduates (29) studied in the Department of Aeronautical Engineering at their final year in 2015. The data related to studying patterns were collected using a self-administered questionnaire which includes 30 questions in the form of four point Likert. The objectives of this study were to evaluate the studying patterns of Aeronautical Engineering undergraduates who studied at their final year in the university and compare the influence of their studying patterns with their Academic class they obtained. A self-administered questionnaire was used to collect the data. Six studying pattern parameters were evaluated in this study. The Academic class achieved by the students who took part in this survey, were also considered for this study after they obtained their Final Grade Point Average (FGPA). The measured studying pattern parameters included reading text books, studying, memorizing, preparing for exams, time management and taking down notes. If the score relating to any study pattern parameter increases, the overall study pattern also increases. When the total points gathered from all study pattern parameters is high, the overall study pattern of that particular student is also getting higher. As per the statistics this scale reported the high level of reliability (internal consistency) as 0.604. In this research, internal consistency of the scale was found as 0.564. Descriptive statistics method was applied at this stage in order to analyze the study pattern of the students. Based on the responses given by the responders, the frequency levels for each question was tabulated in MS Excel worksheet. The Excel worksheet was uploaded to the IBM Statistical Package for the Social Sciences (SPSS) software and each study pattern parameter was evaluated.

It was found that only 31.7 % of the studied group effectively practice the habit of reading text books and only 39.3 % effectively engage in studying. 33.8 % from the whole population practice effective memorizing methods while 56.6 % of students prepare for exams well in advance. Moreover, only 26.9 % of students practices effective time management practices but only 32.4 % of students were good in effective note writing skills. As these studying skills are inefficiently used by majority of the students only 7% of the students from the total were obtained First Class degrees

while the rest could get only general passes.

Keywords: Study patterns, Aeronautical Engineering, Undergraduates

## *INTRODUCTION*

Researchers have recognized several factors of students which influence for their better performances in academic studies [1]. Some of these factors include students' perception about the teaching environment and the teaching strategies of the teacher [2]. One of the main reasons for student failures are due to inadequacy in the skills and attitudes towards their study [3]. Study patterns consider as one of the prominent factors in academic achievements [4, 5]. These studying patterns can be evaluated under several parameters such as regular study, study space, efficient in reading, listening to lectures, active participation for the lessons, doing homework, efficient writing of notes, preparation for exams and attending for exams [6, 7]. In addition, these efficient studying patterns can be explained under major three categories namely, motivation, time management and preparing for examinations [8]. Motivation plays a positive role in students' success academically by enabling them to manage time effectively. It was found that better time management skills lead to reach their goals at the end of the course [9]. Examinations plays an important role in the evaluation process of the students. Hence, effective studying strategies are vital to illustrate the real potential of the students [10]. As per the literature it is obvious that the contribution to enhance the students' effective studying patterns are mandatory in order to improve the university education system as a whole [2]. Further, there are many research articles where the researchers correlated the studying skills and academic achievements [11]. The objectives of this study were to evaluate the studying patterns of aeronautical engineering undergraduates who studied at their final year and compare the influence of their studying patterns for their Academic class they obtained.

## *METHODOLOGY*

In this research, the sample consisted of all the undergraduates (29) studied in the Department of Aeronautical Engineering at their final year in 2015. The data related to studying patterns were collected using a self-administered questionnaire which includes 30 questions in the form of four point Likert. The 30 questions were divided for 6 sections. Each section consists of five questions and those five questions were related to one specific studying pattern parameter. Hence, there were 6 studying pattern parameters and collectively they described the overall studying pattern of undergraduates.

| Question No | Reading Text Books | Response |
|---|---|---|
| 1 | Browsing the headings, chapter questiones before start reading a chapter | |
| 2 | Making questions based on the chapter what I read | |
| 3 | Clarifying the meaning of the new words when I am coming across while reading | |
| 4 | Looking for familier concepts in the chapter | |
| 5 | Looking for the main ideas of the chapter | |
| | **Studying** | |
| 6 | Prefer to study in a quiet and calm environment with less distractions | |
| 7 | Prefer to study long hours while taking short breaks in between | |
| 8 | Keeping all the necessary stationeries with me, while I am studying | |
| 9 | Setting aims while studying such as no of pages read or no of problems solved | |
| 10 | Studying at least two hours per each day in addition to regular lecture hours | |
| | **Memorizing** | |
| 11 | Studying during my personal peak time of energy in order to maintain concentration towards studies | |

| | | |
|---|---|---|
| 12 | Quizzing myself by predicting the subject matters which may tend to appear in future exams or quizzes | |
| 13 | Out loud the difficult concepts in order to understand them better | |
| 14 | Use my own terminology in my lecture notes | |
| 15 | Try to make a link between subject matters which I know already and what is new | |
| | **Preparing for exams** | |
| 16 | Studying with a peers or with a group | |
| 17 | Getting help from lectures or friends to understand what I could not understand during the lecture | |
| 18 | Completing all the assignments on time | |
| 19 | Identifying what I know and what I don't know before I sit for the exam | |
| 20 | Predicting possible questions which could appear in the exam paper and getting ready with the answers | |
| | **Time management** | |
| 21 | Note down upcoming academic activities | |
| 22 | Maintaining a "to do" list to keep track on academic work | |
| 23 | Readying for the upcoming exams well in advanced | |
| 24 | Initiate to work on assigned assignments or projects once they assign those | |
| 25 | Having enough time for fun activities | |
| | **Taking down notes** | |
| 26 | Taking notes while I am reading text books | |
| 27 | Taking notes during lectures | |
| 28 | Rewrite the notes after the lecture | |
| 29 | Compare my lecture note with peer's notes to find out missed subject matters | |
| 30 | Organize the main ideas of the subject matters in a meaning way while writing the lecture note | |

| **Likert scale** | **Never - 0** | **Rarely-1** | **Sometimes -2** | **Often-3** |
|---|---|---|---|---|

The Academic class achieved by the students who took part in this survey, were also considered for this study after they obtained their Final Grade Point Average (FGPA). The measured studying pattern parameters were such as Reading text books, Studying, Memorizing, Preparing for exams, Time management and Taking down notes. If the score relating to any studying pattern parameter increases, the overall studying pattern also increases. The total points gathered from all studying pattern parameters is higher implies that the overall studying pattern of that particular student is high. This scale reported the high level of reliability (internal consistency) as .604. In this research, internal consistency of the scale was found as .564.

TABLE II. ERELIABILITY STATISTICS

| Cronbach's Alpha | Cronbach's Alpha based on standardized items | No of items |
|---|---|---|
| .564 | .604 | 30 |

Descriptive statistics method was applied at this stage in order to analyze the studying pattern of the students. Based on the responses given by the responders, the frequency levels for each question was tabulated in MS Excel worksheet. The Excel worksheet was uploaded to the IBM Statistical Package for the Social Sciences (SPSS) software and the each studying pattern parameter was evaluated.

## *RESULTS AND DISCUSSION*

The generated results for each studying pattern parameters were tabularized and analyzed as follows.

A. Responses related for Reading text book

TABLE III

READING TEXT BOOKS

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| 1.1 | 0 | - | - | - | - |
| | 1 | 7 | 24.1 | 24.1 | 24.1 |
| | 2 | 17 | 58.6 | 58.6 | 82.8 |
| | 3 | 5 | 17.2 | 17.2 | 100.0 |
| | Total | 29 | | | |
| 1.2 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 18 | 62.1 | 62.1 | 65.5 |
| | 2 | 8 | 27.6 | 27.6 | 93.1 |
| | 3 | 2 | 6.9 | 6.9 | 100.0 |
| | Total | 29 | | | |
| 1.3 | 0 | - | - | - | - |
| | 1 | 2 | 6.9 | 6.9 | 6.9 |
| | 2 | 11 | 37.9 | 37.9 | 44.8 |
| | 3 | 16 | 55.2 | 55.2 | 100.0 |
| | Total | 29 | | | |

| | | | | | |
|---|---|---|---|---|---|
| 1.4 | 0 | - | - | - | - |
| | 1 | 3 | 10.3 | 10.3 | 10.3 |
| | 2 | 14 | 48.3 | 48.3 | 58.6 |
| | 3 | 12 | 41.4 | 41.4 | 100.0 |
| | Total | 29 | | | |
| 1.5 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 2 | 6.9 | 6.9 | 10.3 |

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| | 2 | 15 | 51.7 | 51.7 | 62.1 |
| | 3 | 11 | 37.9 | 37.9 | 100.0 |
| | Total | 29 | | | |

Table III, represent the frequencies related to Reading Text Books of the selected sample. Out of 29 students in the sample, there are 7 students who rarely browse the headings, pictures and also the chapter questions before start reading the chapter and there are 17 students who do it sometimes. But there are 5 students, who browse the headings, pictures and chapter questions frequently prior to read the chapter. There is a student who never makes questions from the chapter and there are 18 students who rarely make questions from the read out chapter. Out of 29 students there are 8 students who make questions from the chapter sometimes in order to understand the content of the chapter properly, while there are 2 students who make questions frequently from the read out chapters. In addition, there are 2 students who rarely find out the meaning of new words while reading, where as there are 11 students who find out the meaning of new words sometimes. But there are 16 students who frequently find out the meaning of new words that they come across during reading. There are 3 students who rarely look for similar concepts that they knew already while reading the chapters and there are 14 students who look for familiar concepts in the chapters sometimes. Out of 29 students there are 12 students who frequently look for familiar concepts while reading new chapters. Further, there is a student who never looks for main idea in the chapter but there are 2 students who look for main idea of the chapter rarely while reading. There are 15 students who look for main idea of the chapter sometimes and there are 11 students who try to grab the main frequently out of 29 students.

*B. Responses related for Studying*

TABLE IV

STUDYING

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| 2.1 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 3 | 10.4 | 10.4 | 13.8 |
| | 2 | 13 | 44.8 | 44.8 | 58.6 |
| | 3 | 12 | 41.4 | 41.4 | 100.0 |
| | Total | 29 | | | |
| 2.2 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 6 | 20.7 | 20.7 | 24.2 |
| | 2 | 9 | 31.0 | 31.0 | 55.2 |
| | 3 | 13 | 44.8 | 44.8 | 100.0 |
| | Total | 29 | | | |
| 2.3 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 2 | 6.9 | 6.9 | 10.3 |
| | 2 | 4 | 13.8 | 13.8 | 24.1 |
| | 3 | 22 | 75.9 | 75.9 | 100.0 |
| | Total | 29 | | | |
| 2.4 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 6 | 20.7 | 20.7 | 24.2 |
| | 2 | 17 | 58.6 | 58.6 | 82.8 |
| | 3 | 5 | 17.2 | 17.2 | 100.0 |
| | Total | 29 | | | |
| 2.5 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 9 | 31.0 | 31.0 | 34.4 |
| | 2 | 14 | 48.3 | 48.3 | 82.8 |
| | 3 | 5 | 17.2 | 17.2 | 100.0 |
| | Total | 29 | | | |

As per the data given in Table IV, an out of 29 students there is a student who do not concern about the calmness of the surrounding environment during studying. But 3 students rarely concern about the distractions during studying and there are 13 students who concern about the quietness of the environment sometimes while studying, as well there are 12 students who often concern about the quietness and less distractions of the studying environment. Furthermore, there is a student who studies continuously for lengthy hours with no breaks and there are 6 students who rarely take short breaks during lengthy studying hours. There are 9 students who take short breaks sometimes during lengthy hours of studying. Out of 29 students, 13 students frequently get short breaks during lengthy hours studying. Moreover, there is a student who does not bother about having stationeries during studying and 2 students are rarely concern about having stationeries while studying and there are 4 students who sometimes concern about the having stationeries with them during studying period. In addition, there are 22 students who frequently concern about having stationeries during studying period. Further, there is a single student who never set an aim regarding no of pages to be completed or no of questions to be solved prior to start studying but there 6 students who set aims rarely regarding no of pages to be completed or no of questions to be solved. There are 17 students who set aims sometimes and also there are 5 students who often set aims for no of pages to be completed or no of questions to be completed prior to start studying. Also, there is a student who never studies at least two hours per day in addition to the regular lectures, but there are 9 students who rarely study at least two hours per day in addition to the regular lecture hours. Moreover, there are 14 students who study at least two hours per day sometimes and also there are 5 students who frequently study at least two hours per day in addition to the regular lecture series.

*B. Responses related for Memorizing*

TABLE V

MEMORIZING

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| 3.1 | 0 | - | - | - | - |
| | 1 | 4 | 13.8 | 13.8 | 13.8 |
| | 2 | 20 | 69.0 | 69.0 | 82.8 |
| | 3 | 5 | 17.2 | 17.2 | 100.0 |
| | Total | 29 | | | |
| 3.2 | 0 | - | - | - | - |
| | 1 | 6 | 20.7 | 20.7 | 20.7 |
| | 2 | 19 | 65.5 | 65.5 | 86.2 |
| | 3 | 4 | 13.8 | 13.8 | 100.0 |
| | Total | 29 | | | |
| 3.3 | 0 | - | - | - | - |
| | 1 | 5 | 17.2 | 17.2 | 17.2 |
| | 2 | 14 | 48.3 | 48.3 | 65.5 |
| | 3 | 10 | 34.5 | 34.5 | 100.0 |
| | Total | 29 | | | |
| 3.4 | 0 | - | - | - | - |
| | 1 | 3 | 10.3 | 10.3 | 10.3 |
| | 2 | 4 | 13.8 | 13.8 | 24.1 |
| | 3 | 22 | 75.9 | 75.9 | 100.0 |
| | Total | 29 | | | |
| 3.5 | 0 | - | - | - | - |
| | 1 | 6 | 20.7 | 20.7 | 20.7 |
| | 2 | 15 | 51.7 | 51.7 | 72.4 |
| | 3 | 8 | 27.6 | 27.6 | 100.0 |
| | Total | 29 | | | |

As given in Table V, there are 4 students, who rarely bother about their personal peak time of energy in order to maintain proper concentration level during studying. But there are 20 students who concern about their personal peak time of energy sometimes and try to study during that time. There are 5 students who often concern about their personal peak time of energy in order to memorize subject matters more effectively. There are 6 students who rarely quiz themselves regarding the possible subject matters which can appear in exam papers. In addition, there are 19 students who project quiz questions to themselves sometimes and the rest of 4 students who often project quiz to themselves which can appear in future exam papers. Additionally, there are 5 students who rarely out loud difficult concepts for better understanding while there are 14 students who out loud difficult concepts sometimes in order to understand them effectively. In addition, there are 10 students who frequently out loud difficult subject matters in order to understand them properly. Further, there are 3 students who use their own wordings in order to have a better lecture note while there are 4 students who use their own terminologies sometimes in their own lecture notes. But out of 29 students, 22 students use their own terminologies in their lecture notes for better understanding. Besides there are 6 students who rarely try to create an association between new subjects' matters and the subject matters they already know, while there are 15 students who try to create a link between subject matters sometimes. But there are 8 students who frequently try to create an association between new subject matters and subject matters they already know.

*b Responses related for Preparing for exams*

TABLE VI

PREPARING FOR EXAMS

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| 4.1 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 5 | 17.2 | 17.2 | 20.7 |
| | 2 | 10 | 34.5 | 34.5 | 55.2 |
| | 3 | 13 | 44.8 | 44.8 | 100.0 |
| | Total | 29 | | | |
| 4.2 | 0 | - | - | - | - |
| | 1 | 2 | 6.9 | 6.9 | 6.9 |
| | 2 | 7 | 24.1 | 24.1 | 31.0 |
| | 3 | 20 | 69.0 | 69.0 | 100.0 |
| | Total | 29 | | | |
| 4.3 | 0 | - | - | - | - |
| | 1 | 3 | 10.3 | 10.3 | 10.3 |
| | 2 | 6 | 20.7 | 20.7 | 31.0 |
| | 3 | 20 | 69.0 | 69.0 | 100.0 |
| | Total | 29 | | | |
| 4.4 | 0 | - | - | - | - |
| | 1 | 1 | 3.4 | 3.4 | 3.4 |
| | 2 | 12 | 41.4 | 41.4 | 44.8 |
| | 3 | 16 | 55.2 | 55.2 | 100.0 |
| | Total | 29 | | | |
| 4.5 | 0 | - | - | - | - |
| | 1 | 1 | 3.4 | 3.4 | 3.4 |
| | 2 | 15 | 51.7 | 51.7 | 55.2 |
| | 3 | 13 | 44.8 | 44.8 | 100.0 |
| | Total | 29 | | | |

As according to Table VI, there is a student who does only self-studies and there are 5 students who rarely study as a group with peers. Moreover, there are 10 students who study in a group sometimes and the rest 13 students study in a group frequently with peers. There are 2 students who rarely get the assistance from either

lecturers or friends to clarify difficult subject matters while 7 students are sometimes get the help from lectures or friends to clarify unclear subject matters and the rest 20 students are frequently get the help from lectures or friends to understand subject matters more. Besides, there are 3 students who rarely complete the assignment on time while there are 6 students who complete their assignment sometimes on time. But out of 29 students, there are 20 students who complete their assignments on time. Further, there is a student who never concern about what knows and what does not know in subject matters before taking the exam. But there are 12 students who concern about what knows and what do not know in subject matters sometimes prior to the exam and the rest 16 students are frequently concern about what knows and what do not know before they take the exam. In addition, there is a student who rarely anticipates the possible questions which could appear in the question paper and prepare with the answers while there are 15 students who anticipate the probable questions in exam paper sometimes and prepare with the answers. The rest 13 students are frequently anticipating the possible questions which could appear in the exam paper and get ready with the answers.

*B Responses related for Time management*

TABLE VI I

TIME MANAGEMENT

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| 5.1 | 0 | - | - | - | - |
| | 1 | 20 | 69.0 | 69.0 | 69.0 |
| | 2 | 6 | 20.7 | 20.7 | 89.7 |
| | 3 | 3 | 10.3 | 10.3 | 100.0 |
| | Total | 29 | | | |
| 5.2 | 0 | - | - | - | - |
| | 1 | 11 | 37.9 | 37.9 | 37.9 |
| | 2 | 10 | 34.5 | 34.5 | 72.4 |
| | 3 | 8 | 27.6 | 27.6 | 100.0 |
| | Total | 29 | | | |
| 5.3 | 0 | - | - | - | - |
| | 1 | 6 | 20.7 | 20.7 | 20.7 |
| | 2 | 14 | 48.3 | 48.3 | 69.0 |
| | 3 | 9 | 31.0 | 31.0 | 100.0 |
| | Total | 29 | | | |
| 5.4 | 0 | - | - | - | - |
| | 1 | 7 | 24.1 | 24.1 | 24.1 |
| | 2 | 17 | 58.6 | 58.6 | 82.8 |
| | 3 | 5 | 17.2 | 17.2 | 100.0 |
| | Total | 29 | | | |
| 5.5 | 0 | - | - | - | - |
| | 1 | 5 | 17.2 | 17.2 | 17.2 |
| | 2 | 10 | 34.5 | 34.5 | 51.7 |
| | 3 | 14 | 48.3 | 48.3 | 100.0 |
| | Total | 29 | | | |

As according to Table VII, there are 20 students who rarely note down upcoming academic activities while there are 6 students who note down the upcoming academic activities sometimes. In addition, there are 3 students who often write down upcoming academic activities. There are 11 students who rarely maintain a "to do" list to keep track on academic work, while there are 10 students sometimes use a "to do" list to keep tracks on those. There are 8 students who frequently use a "to do" list in order to keep record on academic work. Furthermore, there are 6 students who rarely start studying for the upcoming tests in well in advanced, while there are 14 students who start studying for upcoming tests sometimes in well in advanced. Out of 29 students only 9 students frequently get ready for the upcoming tests in advanced. There are 7 students who rarely start assignments or projects once they

have assigned while there are 17 students who sometimes start assignments or projects just after they have assigned those. Besides, there are 5 students who often start the assignments or projects once they assigned those. Furthermore, 5 students responded that they rarely get time for fun while another 10 students sometimes get time for fun. But there are 14 students who get time for fun frequently.

*B Responses related for Taking down notes*

TABLE VI II

TAKING DOWN NOTES

| Question number | | Frequencies | | | |
|---|---|---|---|---|---|
| | *Valid* | *Frequency* | *Percent* | *Valid Percent* | *Cumulative Percent* |
| 6.1 | 0 | - | - | - | - |
| | 1 | 6 | 20.7 | 20.7 | 20.7 |
| | 2 | 13 | 44.8 | 44.8 | 65.5 |
| | 3 | 10 | 34.5 | 34.5 | 100.0 |
| | Total | 29 | | | |
| 6.2 | 0 | - | - | - | - |
| | 1 | 3 | 10.3 | 10.3 | 10.3 |
| | 2 | 17 | 58.6 | 58.7 | 69.0 |
| | 3 | 9 | 31.0 | 31.0 | 100.0 |
| | Total | 29 | | | |
| 6.3 | 0 | - | - | - | - |
| | 1 | 13 | 44.8 | 44.8 | 44.8 |
| | 2 | 8 | 27.6 | 27.6 | 72.4 |
| | 3 | 8 | 27.6 | 27.6 | 100.0 |
| | Total | 29 | | | |
| 6.4 | 0 | 1 | 3.4 | 3.4 | 3.4 |
| | 1 | 10 | 34.5 | 34.6 | 38.0 |
| | 2 | 11 | 37.9 | 37.9 | 75.9 |
| | 3 | 7 | 24.1 | 24.1 | 100.0 |
| | Total | 29 | | | |
| 6.5 | 0 | - | - | - | - |
| | 1 | 3 | 10.4 | 10.4 | 10.4 |
| | 2 | 13 | 44.8 | 44.8 | 55.2 |
| | 3 | 13 | 44.8 | 44.8 | 100.0 |
| | Total | 29 | | | |

As given in Table VIII, there are 6 students who rarely taking down notes while they are reading text books and 13 students responded that sometimes they are taking down notes while reading the text books. Out of 29 students, 10 students responded that they use to taking down notes frequently, when they read text books. In addition, there are 3 students who rarely taking notes during lectures and there are 17 students who use to taking down notes at the lecture. But there are 9 students who frequently write down notes when they are in the lecture. Moreover, there are 13 students who rarely rewrite their notes after the lecture and there are 8 students in each category where they rewrite their lecture notes sometimes and more often. Besides, there is a student who never compares the lecture notes with peer's notes to find out missed subject matters, while there are 10 students who do so rarely. But there are 11 students who compare their lecture notes with peers sometimes and there are 7 students who compare their lecture notes with peers more often in order to have a complete lecture note. Furthermore, there are 3 students who rarely organize the main ideas of the subject matters in a meaning way while writing the lecture note. But there are 13 students in each category where they organize the main ideas of the subject matters in a meaning way while writing the lecture note sometimes and more frequently.

*B. Responses related for achieved Academic Class*

TABLE IX

TOTAL AVERAGE OF THE EACH MAXIMUM RESPONDERS PERCENTAGE FOR EACH TESTED STUDYING PATTERN PARAMETER

| Studying pattern parameter | No of respondents | Total Average of the each Maximum responders percentage |
|---|---|---|
| Reading text books | 29 | 31.7 |
| Studying | 29 | 39.3 |
| Memorizing | 29 | 33.8 |
| Preparing for exams | 29 | 56.6 |
| Time management | 29 | 26.9 |
| Taking down notes | 29 | 32.4 |

As according to the Table IX, only 31.7 % of the responders practices Reading text books and only 39.3 % practice effective studying methods. Besides, only 33.8 % of the respondents use effective memorizing techniques while only 56.6 % prepare for exams in an effective way. Proper time management techniques practice by 26.9 % from the studied group and only 32.4 % of students taking down notes in effective manner.

*C. Responses related for achieved Academic Class*

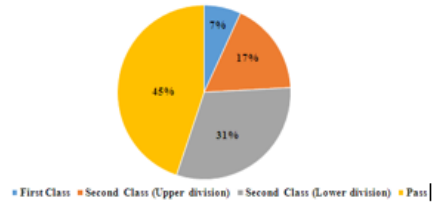


Fig. 1.Academic Class achieved by the responders

As shown in Fig.1, from the studied group of students only 7 % could obtained First Class and only 17 % of students could obatined Second Class (Upper division) as their academic achievements. Further, 31 % of the students from this sample could achieved Second Class (Lower division) while the rest 45 % of students could achieved Pass as their academic performances.

## *CONCLUSIONS*

Based on the results of this study, it is clear that only 31.7 % from the studied group had followed most effective studying pattern in Reading text books while only 39.3 % from the studied group followed most effective study pattern for Studying. Out of the whole population, only 33.8 % were used proper memorizing methods and only 56.6 % of students followed most effective methods when it comes for preparing for exams. But from the studied group only 7 % could achieved First Class while another 17 % could achieved Second Class (Upper division). The most effective time management techniques were followed only by 26.9 % of the students and only 32.4 % were effectively take down notes. Hence, 31 % from the studied group could obtained Second Class (Lower division). The studied group of students were practicing ineffective studying patterns which led them to obtained lower grades. Hence, the majority of the students could obtain Pass grade as their final academic achievement. The poor academic performances of the students coincide due to practicing of ineffective studying patterns. Hence, it is important to encourage students to practice most effective studying pattern in order to obtain highest academic performances.

**Acknowledgment**

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# IDENTIFYING THE CHALLENGES OF BIG DATA USAGE IN THE EDUCATIONAL SECTOR OF SRI LANKA

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## *ABSTRACT*

The volume and velocity of data generated have increased substantially in the education sector of Sri Lanka. Although there is an increase, the majority of educational employees do not realize the importance or value of data and often discard without taking into consideration the numerous benefits big data can offer to students and educational institutions. The root causes that contribute to the lack of utilization of big data are mainly due to lack of infrastructure, trained workforce, standards of data usage, security issues, policies, and education of employees. The main objective of this study is to identify the challenges faced by the education sector of Sri Lanka when dealing with big data implementation and further recognize the incentives that encourage the use of big data to support the development, profitability, and decision-making capabilities of Sri Lanka. Qualitative data will be collected from interviews taken from managers, marketing professionals, accountants, and lecturers in various educational institutions across Sri Lanka using snowball and convenience sampling techniques. Furthermore, to establish how educational employees use big data, a survey will be distributed to various educational employees working in different regional educational institutions across Sri Lanka. SPSS is used to determine the correlation between independent variables (infrastructure, trained workforce, policies, education, security, and standards) and dependent variable (data usage). The findings of this research would help educational employees understand the value of big data. Moreover, this research would contribute to improving the profitability and standards of decision-making processes in the education sectors of Sri Lanka.

Keywords: Big data, data usage, education sector, Sri Lanka, data velocity

## *INTRODUCTION*

In today's society, big data is a commonly discussed topic due to the availability and use of data for various purposes in different industries. Big data can be defined in simple terms as large volume of data which are organized and unorganized. Having ample amount of data does not matter for any industry, if there is lack of usage from it. Big data can be used to identify the market and create better strategic decisions that lead to competitive advantage of the business. An industry analyst called Laney (2001) identified three dimensions and properties of big data, namely Volume, Velocity and Variety. Volume is referred to as the amount of data that can be collected from variety of sources which include from daily business transactions, social media activities, data collected from sensor or from data transferred from one machine to another (Laney, 2001). In the past storing

of these huge volume of data would be a great difficulty, but nowadays open source software like Apache hadoop have provided a software framework for storing ample amount of data conveniently. It is expected to increase the data storage by 300 times from 2005 to 2020 which is 43 trillion gigabytes. Most of the companies in the world have a storage capacity of more than 90 Tb of data nowadays.

Nevertheless, velocity can be referred to as data processing speed. It is unbelievable that the way sensors, radio frequency identification and smart metering devices capture information and process them at rapid pace. It is identified that New York stock exchange captures 1TB of trade information daily. Even a vehicle that runs on the road has more than 100 sensors to capture data and drive away from traffic efficiently (Laney, 2001). Finally, variety can be defined the amount of different data available. Data can come in different formats, for instance one format is unstructured data such as financial data, stock ticker, audio, video data and text documents whereas another format is structured data such as numeric data in traditional databases.

In addition to the above mentioned Dong Lanely data classification, two other data dimensions are identified recently. They are Variability and Complexity. Variability means that with the increase of amount of variety of data the data flow can differ by maybe having peaks or by falls (McNulty, 2016). For instance, seasonal data received in online shopping sites tend to differ significantly. Finally, Complexity refers to data that comes from different sources tend to be difficult to managed and linked (Khan et al, 2014). Most difficult task today is to identify the connection that is having certain type of data. Data analyst spend plenty of time sorting these data and making it meaningful to the relevant parties. Data types can be structured, semi structured and unstructured data. This has to be analyzed and sorted in order to make them meaningful.

With the volume, verity, velocity, variability and complexity of data it is difficult to understand what we are going to do with it (McNulty, 2016). Most of the companies in various industries have used these data to take advantages such as cost reduction, new product development, time reduction and smart decision-making, but ample amount of data go to waste without any usage. Although most industries use big data to receive comparative advantage, education sector of Sri Lanka tend to show a negative attitude towards big data usage. Education sector is where there is so many skilled professionals such as doctors, accountants, engineers, mechanics and businessman are made, but attention of big data use seems to be greatly less. In education industry, there is currently a regular interaction of students with the technology. As a result of regular interaction, most of the big data created in educational industry are student assignments records uploaded to the Moodle, teachers notes uploaded to the system, metric systems to monitor students' attendance and performance, digital libraries to issue and return books as well as entrance and graduation records of students (Shacklock, 2016). Each of these mentioned data records is responsible for bringing in many students and developing the education sector in the economy. Big data usage can enable education institutes to retain students, improve the graduation rates, improving quality of the teaching and curriculum (Economist Report, 2008). Furthermore, big data would help to receive necessary funding to archive organizational efficiency (Petkovics et al, 2014). With all the above mentioned benefits, value of big data is not understood by the educational sector employees in Sri Lanka. They tend to discard and ignore the data available without getting proper usage of the data. By realizing that there is a problem to

clarify and verify the availability of problem, pilot study was conducted with 20 employees in education sector of Sri Lanka. The study was done through telephone interview and discussion using convenient and snowball sampling where mostly open-ended questions were asked from participants.

From the Pilot study, it was identified one of the major root cause for ignoring big data is lack of infrastructure. Most of the places in Sri Lanka lacks telecommunication network to link and connect to the required networks to share information. Moreover, special servers and multiple processors are required to handle and store the educational data, but due to the cost factor most of the educational institutes in Sri Lanka cannot afford the servers and processors. Secondly, it was identified that in order to use big data, there should be trained and skilled workforce. These workforce should be aware of the current technological development and should process developer skill set. Not only IT knowledge they also should possess the ability to analyze data using statistics and mathematics. To find the appropriate skills required in the education industry is especially a huge challenge. Only few possess all round skill set and others even lacks ICT knowledge. Therefore, to adopt and use big data is a major challenge in the education industry.

Thirdly, ample amounts of data are captured regularly by the student databases but there is no proper standards of maintaining these data. All the data are stored in one place without any categorization of data. When a particular need arises educational administrators find it difficult to retrieve the data that is relevant to the issue. As a result most of educational administrators tend to discourage the use of big data. Additionally, security issues are a major challenge to the professionals in the educational industry. Mainly, because antivirus software that is need to protect data need regular updating and need up to date protection. If the data protection malfunction the whole system can bring down to a standstill position and hackers could use those information to their own advantage. To purchase and update these virus guards to protect the whole databases in education sector also cost so much money. Therefore, due to limited budget and complexity educational administrators consider maintain security as a challenge to the big data usage. Most of the policies implemented in Sri Lankan educational institutes does not support the big data usage. For instance, there is a policy that student exam records are kept on manual books for maximum 5 years, after that they are considered no longer useful. If a particular student require those details after 5 years destroyed data cannot be retrieved from the system.

Finally, ICT education literacy level of Sri Lanka is declining from 28.5% in 2017 to 27.5% in 2018 from overall population according to department of census of Sri Lanka (Census, 2018). Lack of computer literacy suggests that lesser education level to cope up with huge databases in the society. Therefore, due to lack of education in ICT is a challenge to the use and implementation of big data in education sector in Sri Lanka. It could be seen that there are many root causes that lead to major problem of lack of big data usage in education industry. Neglecting without taking any necessary precautions for improvement of big data usage would impact our standards of education with the world and also would make poor decisions in education industry. As a result, of poor decision making it will weaken the profitability of education services in Sri Lanka. Therefore, necessary corrective actions need to be implemented for improvement of big data usage.

This paper would recognize the importance of big data usage to education sector of Sri Lanka and also would

highlight the need for actions to be taken to encourage big data usage. With the finding of this paper most of the professionals in education sector will realize the value of big data usage which in return improve the decision-making, profitability and the competiveness in the education sector of Sri Lanka. Primary objective of this paper is to determine the main challenges of big data usage in education sector Sri Lanka. Secondly, to evaluate and see weather identified Challenges have any correlation with the big data usage. Furthermore, to understand whether the realized challenges would continue to be challenges in future as well. Finally, to recognize the recommend actions to be implemented in order to encourage data usage.

With lack of big data usage becoming a major problem the research would identify what are the main challenges faced by education sector of Sri Lanka of big data implementation? Further, need to determine whether there is a relationship between the identified challenges and big data usage? Additionally, to predict whether identified challenges would contribute to the future big data usage? Finally, this research would try to recognize what are the appropriate recommend solutions in order to encourage big data usage in education industry Sri Lanka?

This paper mainly focuses on answering the above mentioned questions. The boundaries are set forth in order to achieve a more valid and accurate outcome. Research mainly uses managers, marketing professionals, accountants and lecturers in selected areas such as Kurunegala, Kandy and Colombo in Sri Lanka within 8-year time period (2010-2018).

## *LITERATURE REVIEW*

There are various advantages of utilizing big data, however it can be seen that the implementation of big data is a major challenge for the different sectors in one's economy. Hence, empirical evidences from various studies have highlighted the actual and perceived challenges of big data usage in diverse industries. According to Stephen Kaisler et al. (2014), there are numerous issues and challenges of moving forward with big data implementation for education sector as well as some other sectors. Some of the most common challenges that were highlighted are issues of storage, attitudes of management, affordability, issues of processing, not having tools to analyze and also not having powerful algorithms to sort the big data from their systems or processes.

Other different studies conducted by Daniel (2014); Shitut (2017); and Kernochan (2013) also emphasize certain challenges that act as barriers for the implementation of big data systems. Mainly, they have identified such challenges as difficulty of acceptance (most of the management are not willing to change from traditional systems of data analytics), difficulties in accessing relevant data (sorting from ample amount of data and obtaining required data is a problem), obtaining expertise knowledge to operate (lack of relevant skills is a problem) and barriers in the organization environment (linking all the departments for big data implementation is a problem).

Another study by Long and Siemens (2011) demonstrate that not accurate data, mismanagement of data, culture, privacy issues, lack of skills, not enough return on investment, lack of training and resources as well as difficulty in data standardization are major challenges in application of big data in the industry. Further, studies conducted by authors such as Dan and Roger (2010); Jayasree (2013); and Rachana and Guruprasad (2014) debate that security, reliability, data quality, cost,

performance and data storage facilities are key issues in big data implementation in major economies. Some of the major challenges that are identified from different literatures have been discussed below for better understanding.

**Privacy and security**

Most of empirical studies have identified that when it comes to raw data and information, securing and the privacy of data are as a biggest threat for any industry and any organization. When ample amount of data are used for various purposes they should be securely stored in order to prevent it from usage of various third parties. Ferguson (2017) identifies that some data are valuable for making vital decisions in the organization, but there are possibility of targeted cybercrimes and hacking of such valuable data due to competitive edge and various other reasons. Eynon (2013) also suggests that fear of misuse of valuable data lead to lack of implementation of big data in most of the companies. Additionally, a comprehensive study conducted by Broeders et al. (2017) also state that misuse of sensitive data and information fraud is a major concern in big data utilization. Hence, it is evident that majority of organizations have fear of using big data due to the lack of privacy and security of valuable data and information.

**Infrastructure**

Infrastructure is a vital component in big data usage, especially in today's society. According to Barroso, Clidaras and Hölzle (2013) telecommunication is considered as a vital infrastructure or a factor for implementing big data projects. In order to connect with big data storage systems such as cloud systems, telecommunication systems are considered highly necessary for any organization. Furthermore, specially designed architecture is required to process millions of nodes with multiple of disks and processors at high internet connection speed (Shapiro and Varian, 2010). Moreover, maintaining all the infrastructure leads to number of side effects such as rise of huge costs and additional resources and support systems. Hence, only large companies have the necessary capacity to bear those costs and other required resources (Barroso, Clidaras, and Hölzle, 2013). Therefore, obtaining necessary infrastructure to implementing big data usage is a massive challenge in any industry.

**Trained workforce**

According to Hilbert (2013), proper adaptation is required to big data project implementation. For a proper adaptation of big data software, it is necessary to acquire trained workforce within the organization. He also suggests that if data is distributed in several clusters, expertise knowledge can be obtained from several communities as well. For instance, companies like Google and Facebook use open source software like Apache hadoop to distribute their data among several clusters and obtain necessary expertise knowledge (Hilbert, 2013). Hence, it can be seen that it is essential to have a trained workforce with some expertise knowledge in order to implement the big data systems and processes in organizations.

**Education level of employees**

According to Villars et al. (2011), it is crucial to acquire adequate knowledge of information technology and application developing skillset in order to effectively apply big data systems and processes. People who have the relevant knowledge are scares and difficult to acquire today in terms of information technology, especially when it comes to developing countries like Sri Lanka. Moreover, studies piloted by Manyika et al (2011) suggest that key subject areas such as statistics, mathematics and computer science are needed to be enhanced regularly by employees in order effectively integrate with big data implementation. His study prove that

having lack of knowledge in the mentioned subject areas would make employees difficult to analyze and interrupt the big data and data would be meaningless to conduct the further business operations. Thus, in order to implement the big data systems successfully, organizations should have right skillset and knowledgeable employees who can be identified as difficult to acquire in today's job market.

Policies and standards of data usage

Majority of empirical evidences have identified another challenge of implementing big data systems which is lack of policies and standards of data utilization. Protecting data privacy in health services report (2000) stresses out the necessity of collecting data in a standard procedure, mainly because of big data projects are vulnerable for possible security threats. Report also elaborates that strategies should be implemented by companies as a part of their policy guidelines and code of conduct in order of methods data should be collected, how should be protected and the procedure they should be used. Moreover, the action plan needs to be constructed in order of how data should be managed in order to protect from possible security breaches and data losses. In addition, Campbell (2007) identifies regulatory framework is necessary to protect against possible data breaches and security losses and also would create trust among the users of big data, if there is proper regulatory procedures.

## *THEORETICAL FRAMEWORK OF BIG DATA USAGE*

Technology Acceptance Model (TAM) which was developed by Davis in 1989. This theory determines that new technology is mainly accepted by users due to perceived usefulness and the ease of use. When people perceive that new technology is not beneficial for them and complicated to use they tend to neglect the acceptance of usage of new technology (Davis, 1989). Therefore, from this theory we can deduce that perceived usefulness and ease of use contribute to acceptance of big data usage and implementation in organizations.

Technology Task Fit Model (TTF) assumes that user require necessary technology that fit their working environment to increase employee performance as well as organizational performance. When user lacks the necessary equipment, they find it difficult to perform to the expected standards by the organization which creates a visible gap of expected performance and actual performance. So in that sense, the performance is heavily correlated with the appropriateness of technology (Goodhue, and Thompson, 1995). Therefore, it can be recognized from technology task fit model that employees require necessary equipment in order to boost their actual performance levels to be successfully implement the big data systems within their working environment. Theory of Planned Behavior (TPB) which was developed by Icek Ajzen in 1988 which predicts the behavioral intention of humans to make decisions. Main factors that lead to behavior intention are attitudes, subjective norms and perceived behavioral control. People tend to make positive decisions based on agreement of all the factors mentioned (Ajzen, 1988). For instance, if people have positive attitudes, cultural views tend to support and also if they believe it is easy to use, then people tend to accept new technologies, particularly like big data processes. Hence, it is vital to understand the perception and intention of management as well as employees when implementing the big data processes and systems in the organization.

Unified Theory of Acceptance and Use of Technology (UTAUT) is a developed

theory that discusses the intention to use technology in any industry (Venkatesh et al, 2003). This theory identifies behavioral intention of people to use the different systems is mainly driven by expectancy of the performance of the system, amount of effort, influences by the society and conditions that support the system. Thus, a study by Venkatesh et al. (2003) state that people's behavioral intention to use the system diminishes, if any of the conditions mentioned do not provide the necessary facilitation. Therefore, it can be identified that there has to be a clear and proper intention of implementing big data systems in every industry as well as the people who relates to it should be clearly understood these intentions to productively utilize the big data in their daily operational activities.

Motivation model (MM) theory was introduced in 1992 by Davis, Bagozzi and Warshaw in order to identify the adaptation and utilization of information and communication technology within an organizational environment. They stress out that usage is mainly depend on intrinsic and extrinsic motivators (Davis et al, 1992). Intrinsic motivators arises from person's inner motive to perform a task, for example satisfaction received from the computer usage is an intrinsic motivator. Extrinsic motivator arises from outside of a person, for instance factors from the society, perceived benefits and usefulness encourages people to use technology (Davis et al, 1992). Therefore, it can be determined that according to this theory intrinsic and extrinsic factors motivates people to use and implement big data for their daily operations.

Model of PC Utilization discusses about leading factors that lead to utilization of technology. Main factors such as social factors, complexity, job fit, long term consequences, affect towards PC usage and fascinating conditions greatly affect technology usage (Thompson et al, 1991). According to this study by Thompson et al (1991), they believe that people tend to absorb and adopt technology based on certain factors such as; if the social factors support, technology is less complex to use, day-to-day tasks fit their working environment, less consequences of personal computer usage and there should be supporting conditions to use personal computers. Hence, it could be understood all these six factors can contribute the big data usage and implementation in an organization.

## *METHODOLOGY*

Based on the analysis of literature review and pilot study conducted, this research investigates whether the identified challenges can impact the big data usage in the education sector of Sri Lanka. Consequently, big data usage in education sector in Sri Lanka is considered as dependent variable and lack of infrastructure, trained workforce, policies, ICT education and lack of security is identified as independent variables. The research mainly tests weather there is a relationship between the independent and dependent variables and also research tries to predict whether the particular identified connection would continue to the future using regression analysis.

Conceptual framework

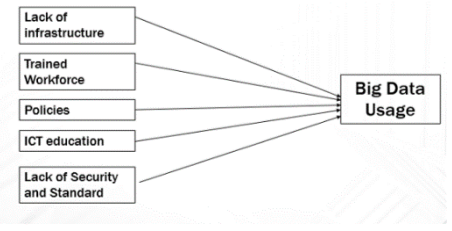


*Figure 1 - Conceptual Framework*

**List of Hypothesis**

H0: There is no relationship between Infrastructure and big data usage in Sri Lanka.

H1: There is relationship between Infrastructure and big data usage in Sri Lanka.

H0: There is no relationship between trained workforce and big data usage in Sri Lanka.

H2: There is a relationship between trained workforce and big data usage in Sri Lanka.

H0: There is no relationship between policies and big data usage in Sri Lanka.

H3: There is relationship between policies and big data usage in Sri Lanka.

H0: There is no relationship between ICT education and big data usage in Sri Lanka.

H4: There is a relationship between ICT education and big data usage in Sri Lanka.

H0: There is no relationship between security and standards and big data usage in SL.

H5: There is relationship between security and standards and big data usage in SL.

Population, Sample size and sample selection

It was identified that approximately 120,000 private sector employees working in education sector in Sri Lanka. Managers, marketing professionals, accountants, and lecturers in various fields are considered to be working in educational industry in Sri Lanka. From the whole population, 130 employees working in different sectors are selected using simple random sampling technique. Areas such as Colombo, Kandy and Kurunegala are selected for the research depending on the convenience.

Data collection, Questionnaire type, time and Data analyzing technique

Data collection was done using an online questionnaire mainly because it could be easier to reach a widespread population. Selected all 130 employees have access to internet and they could answer the questions in their own spare time. Questionnaire was designed in a simple manner with mostly close-ended questions. Questions are setup using Likert scale that ranges from 1=strongly agree, 2= agree, 3=neither agree nor disagree, 4= disagree and 5=strongly disagree. Questionnaire is designed in a manner to derive at specific answer in the questionnaire. This research is conducted within time frame of 01st October 2018 to 28th February 2019. Data analysis is done using IBM SPSS software to facilitate correlation and regression.

**Reliability test**

Cronbach's Alpha reliability test was conducted in order to measure the complete stability of the outcomes of the statements in the questionnaire involving to the variables highlighted. It was found that more than 90% of data are reliable and satisfactory.

**Reliability Statistics**

| Cronbach's Alpha | Cronbach's Alpha Based on Standardized Items |
|---|---|
| .974 | .978 |

*Table 1 - Reliability Statistics*

## *DATA ANALYSIS*

**Correlation analysis**

Pearson Correlation is used to identify weather there is any relationship between independent and dependent variables. In Pearson correlation, coefficient range determines the type of relationship between variables which further depicts in the following table.

| Coefficient Range | Interpretation |
|---|---|
| 0.90 to 1.0 | Very strong positive correlation |
| -0.90 to -1.0 | Very strong negative correlation |
| 0.70 to 0.90 | High positive correlation |
| -0.70 to -0.90 | High negative correlation |
| 0.50 to 0.70 | Moderate positive correlation |
| -0.50 to -0.70 | Moderate negative correlation |
| 0.30 to 0.50 | Low positive correlation |
| -0.30 to -0.50 | Low negative correlation |
| 0.0 to 0.30 | Negligible correlation |
| -0.00 to -0.30 | |

*Table 2 - Coefficient range for correlation*

## Regression analysis

Simple regression analysis examines when the value of dependent variable Y (big data usage) can be effectively predicted using the Independent variable X (Infrastructure, trained workforce, policies, ICT education and security and standard).

Relationship between Infrastructure on big data usage in Education sector Sri Lanka

**Correlations**

| | | Infrastructure at the educational sector has an effect on big data usage. | There are significant challenges on implementation on big data usage in educational industry |
|---|---|---|---|
| Infrastructure at the educational sector has an effect on big data usage. | Pearson Correlation | 1 | .810** |
| | Sig. (2-tailed) | | .000 |
| | N | 130 | 130 |
| There are significant challenges on implementation on big data usage in educational industry | Pearson Correlation | .810** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 130 | 130 |

**. Correlation is significant at the 0.01 level (2-tailed).

*Table 3 - Correlation between infrastructure and big data usage in Education sector*

Based on the above analysis it indicates that Pearson correlation of r value is 0.810 which implies that there is a high positive correlation between variables. Moreover, Sig. (2-tailed) value is 0.000 which is less than 0.05 this means when one variable increase or decreases another variable also changes significantly. From the hypothesis, it can reject null hypothesis and accept H1: which shows there is a relationship between infrastructure and big data usage. Therefore, since there is a positive correlation, it suggests when there is lack of infrastructure there is lack of big data usage and also when people are provided with proper infrastructure, the big data usage tend to be high.

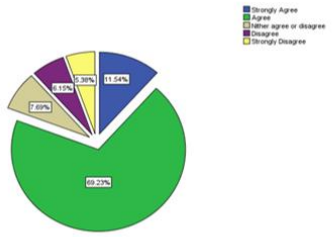


*Figure 2 - Opinions about infrastructure and big data usage in Sri Lankan Education sector*

According to the analysis of survey 69.23% agreed better infrastructure is required for higher big data usage in educational sector. Moreover, 11.53% of educational sector employees disagreed that infrastructure lead to improvement big data usage. Overall, majority agreed that infrastructure is a requirement in big data usage.

Regression analysis infrastructure on big data usage

**Coefficients***

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .765 | .085 | | 9.008 | .000 |
| 1 | Infrastructure at educational sector has an effect on big data usage. | .546 | .035 | .810 | 15.626 | .000 |

a. Dependent Variable: There are significant challenges on implementation on big data usage in educational industry

*Table 4 – Regression on infrastructure and big data usage in Sri Lankan Education sector*

Regression equation to determine Impact for big data usage from infrastructure is determined as follows: Y=0.546X+0.765. Where X is the people viewpoint of the need for infrastructure where Y is the change of big data usage. With the regression equation identified it could be determined that in future people believe proper infrastructure is necessary for big data adaptation.

Relationship between trained workforces on big data usage in education sector

Correlations

| | | ICT Trained workforce at the education sector has effect on big data usage | There are significant challenges on implementation on big data usage in educational industry |
|---|---|---|---|
| ICT Trained workforce at the education sector has effect on big data usage | Pearson Correlation | 1 | .834** |
| | Sig. (2-tailed) | | .000 |
| | N | 130 | 130 |
| There are significant challenges on implementation on big data usage in educational industry | Pearson Correlation | .834** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 130 | 130 |

**. Correlation is significant at the 0.01 level (2-tailed).

*Table 5 - Correlation between trained workforce and big data usage in Education sector*

From the information Pearson R value is 0.834 which implies there is a high positive correlation. Additionally, Sig. (2-tailed) value is .000 which is less than 0.05 which suggest when one variable change other also changes positively. When we consider hypothesis, we can reject null hypothesis and accept H2: There is a relationship between trained workforce and big data usage in Sri Lanka. From all the information it could be determined that trained workforce is necessary for big data implementation.

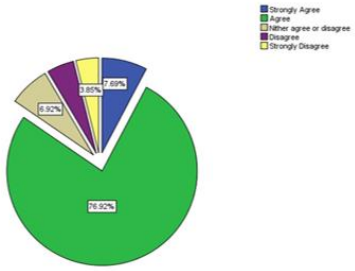


*Figure 3 - Opinions about trained workforce and big data usage in Education sector*

Information from the questionnaire suggest that 76.92% implies that training is required for big data adaptation. Furthermore, only 8.47% disagree that training is necessary for big data implementation. Majority agree that training is a vital for big data implementation.

Regression analysis trained workforce on big data usage

Coefficients[a]

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .552 | .090 | | 6.167 | .000 |
| | ICT Trained workforce at the education sector has effect on big data usage | .655 | .038 | .834 | 17.109 | .000 |

a. Dependent Variable: There are significant challenges on implementation on big data usage in educational industry

*Table 6 – Regression on trained workforce and big data usage in Education sector*

Regression equation to identify whether training is necessary in future for big data usage is determined as follows: Y=0.655X+0.552. From the equation it could be determined that even in the future people believe that trained workforce is necessary for big data usage.

Relationship between applied policies on big data usage in Sri Lankan education

Correlations

| | | Applied Policies in the education sector has effect on big data usage | There are significant challenges on implementation on big data usage in educational industry |
|---|---|---|---|
| Applied Policies in the education sector has effect on big data usage | Pearson Correlation | 1 | .777** |
| | Sig. (2-tailed) | | .000 |
| | N | 130 | 130 |
| There are significant challenges on implementation on big data usage in educational industry | Pearson Correlation | .777** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 130 | 130 |

**. Correlation is significant at the 0.01 level (2-tailed).

*Table 7 - Correlation between policies and big data usage in Sri Lankan Education sector*

From the information it could be determined that Pearson R value is 0.777 which indicates that high positive correlation. Additionally, sig (2-tailed) value is .000 which is lesser than 0.05 this indicates when policies changes big data usage also changes relatively. Moreover, we can reject null hypothesis and accept H3: there is relationship between policies and big data usage in Sri Lanka. Further, it could be determined lack of policies leads to lack of big data usage and also better policies leads to higher big data usage.

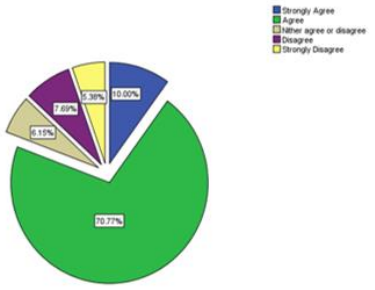


*Figure 4 - Opinions about applied policies and big data usage in Education sector*

Information obtained from the questioner suggest that 70.77% agree that proper policies should be implemented in order for effectively promote big data usage. Moreover, 13.07% disagree that proper policies are necessary for encourage big data usage. Overall, majority decides that proper policies are crucial to encourage big data usage.

Regression analysis policies on big data usage

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .809 | .092 | | 8.834 | .000 |
| | Applied Policies in the education sector has effect on big data usage | .520 | .037 | .777 | 13.981 | .000 |

a. Dependent Variable: There are significant challenges on implementation on big data usage in educational industry

*Table 8 – Regression on policies and big data usage in Sri Lankan Education sector*

From the table above we can arrive at the regression equation as follows: Y=0.520X+0. 809. This regression equation indicates that people believe when applied policies increases big data usage also increases. It also predicts that in future employees believe proper policies is necessary for improve big data adaptation.

Relationship between ICT Education on big data usage in Education sector

**Relationship between ICT Education on big data usage in Education sector**

**Correlations**

| | | ICT Education is required in the education sector for the big data usage | There are significant challenges on implementation on big data usage in educational industry |
|---|---|---|---|
| ICT Education is required in the education sector for the big data usage | Pearson Correlation | 1 | .835** |
| | Sig. (2-tailed) | | .000 |
| | N | 130 | 130 |
| There are significant challenges on implementation on big data usage in educational industry | Pearson Correlation | .835** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 130 | 130 |

**. Correlation is significant at the 0.01 level (2-tailed).

*Table 9 - Correlation between ICT education and big data usage in Education sector*

Pearson R value 0.835 indicates high positive correlation between ICT education and big data usage. Additionally, Sig (2-tailed) value 0.000 is lesser than 0.05 suggests when changes in ICT education impacts on big data usage. It can reject null hypothesis and accept H4: there is relationship between ICT and big data usage. Overall, data indicates that lack of ICT education lead to lack of big data usage and vice-versa.

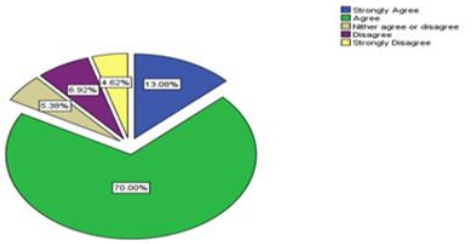


*Figure 5 - Opinions about ICT education and big data usage in Education sector*

70% of respondents agree that ICT education is required to improve big data usage in education sector in Sri Lanka whilst 11.54% disagree on that statement. Overall, majority accepts that education is a requirement for big data usage in Sri Lanka.

Regression analysis ICT education on big data usage

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .735 | .079 | | 9.261 | .000 |
| | ICT Education is required for big data usage in education | .572 | .033 | .835 | 17.173 | .000 |

a. Dependent Variable: There are significant challenges on implementation on big data usage in educational industry

*Table 10 - Regression on ICT education and big data usage in Education sector*

From the regression analysis the equation that can be arrived is: Y=0.572X+0. 735. From the equation it can be predicted that people believe when ICT education increases the big data usage also increases.

Relationship between security and standards on big data usage in Sri Lankan Education sector

**Correlations**

| | | Lack of Security and standards in the education sector has effect on big data usage | There are significant challenges on implementation on big data usage in educational industry |
|---|---|---|---|
| Lack of Security and standards in the education sector has effect on big data usage | Pearson Correlation | 1 | .972** |
| | Sig. (2-tailed) | | .000 |
| | N | 130 | 130 |
| There are significant challenges on implementation on big data usage in educational industry | Pearson Correlation | .972** | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 130 | 130 |

**. Correlation is significant at the 0.01 level (2-tailed).

*Table 11 - Correlation between security and standards on big data usage in Education sector*

Pearson R value is 0.972 which indicates very strong positive correlation between security standards and big data usage. Additionally, Sig. (2-tailed) value is 0.000 which is lesser than 0.05 that suggest security standards improve big data usage also changes positively. Hence, we can reject null hypothesis and accept where H5: There is relationship between Security Standards and big data usage. Finally, analysis depicts lack of higher security and standards leads to Lack of big data usage and also high security and standards leads to higher big data usage.

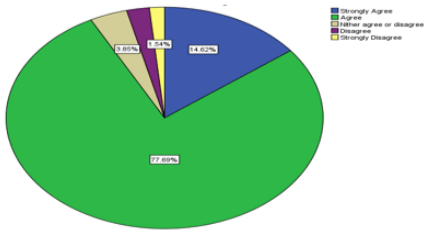


*Figure 6 - Opinions about security and standards on big data usage in Education sector*

Above pie chart illustrates 77.69% agreed that lack of security and standard in the education sector lead to lack of big data usage whereas only 3.84% disagreed that security and standards are essential for big data usage.

Regression analysis of security and standards on big data usage

**Coefficients[a]**

| Model | | Unstandardized Coefficients | | Standardized Coefficients | t | Sig. |
|---|---|---|---|---|---|---|
| | | B | Std. Error | Beta | | |
| 1 | (Constant) | .117 | .042 | | 2.782 | .006 |
| | Lack of Security and standards in education has effect on big data usage | .945 | .020 | .972 | 46.697 | .000 |

a. Dependent Variable: There are significant challenges on implementation on big data usage in educational industry

*Table 12 - Regression on security and standards on big data usage in Education sector*

From the information we can derive at the equation Y=0.945X+0.117. It can be understood from the equation that employees in education sector Sri Lanka believe that in future they might consider security and standards as a necessity for big data usage.

## *DISCUSSION*

This study clearly identified that infrastructure, trained workforce, security standards, policies and education of employees are required for big data utilization. Firstly, it is identified that ICT infrastructures such as computers, processors, storage facilities and network

facilities are considered vital for education sector to use big data facilities. The data clearly supports that without proper infrastructure, big data implementation is difficult by indicating Pearson R value 0.810 and Sig.2 (tailed) value is 0.000 which is less than 0.5. Furthermore, education sector employees in Sri Lanka would consider that infrastructure is a necessity in future for big data usage according to regression equation: Y=0.546X+0.765. Secondly, it is understood that training the workforce in education sector of Sri Lanka is necessary in order to work with big data and also to encourage big data usage. When workers are trained properly, they will understand how to work with appropriate systems effectively without impacting the whole process. With the Pearson R value identified as 0.834 and sig(2-tailed) value 0.000 which is lesser than 0.5 this suggest that there is high positive correlation between train workforce and big data usage. Furthermore, with the regression equation Y=0.655X+0.552, it could be predicted that education sector employees in Sri Lanka feel that training workforce is a must to encourage big data usage. Thirdly, it is recognized that proper policies need to be implemented by government to encourage big data usage among education sector employees in Sri Lanka. For instance, simple rules and policies need to be implemented to data recording, storing, processing and accessing data so that education sector employees would be motivated to use data. From the data analyzed Pearson R value is identified as 0.777 and Sig (2-tailed) value 0.000 this implies that there is high positive correlation between policies and big data usage. Moreover, education sector employees believe that in future they need simple and supportive policies to encourage big data usage according to regression equation: Y=0.520X+0.809. Moreover, ICT knowledge and skills in Sri Lanka is considered at a lower level, hence it will heavily impact on managing big data. From the information analyzed Pearson R value is at 0.835 and Sig (2-tailed) value is 0.000 which suggest high correlation between ICT education and big data usage in Sri Lanka. This implies knowledge and skills are highly required to implement big data. Even for the future education sector employees will consider knowledge and skills as a requirement for them to implement big data. Finally, education sector employees in Sri Lanka believe that security and standards are a necessity and constantly need to be updated in protecting systems against harmful viruses to effectively implement big data usage. According to opinions given, there is tendency of stealing valuable data and also misuse of data so security and standards are considered highly important for big data usage in Sri Lanka. This is clearly suggesting that from the analyzed data where it shows Pearson R value 0.972 and Sig (2-tailed) value 0.000. Most importantly, according to the regression equation Y=0.945X+0.117 education sector employees perceive that security standards are necessary for big data usage in the future as well.

## *CONCLUSION*

This research mainly focuses on identifying challenges that impact on big data usage in education sector in Sri Lanka. By referring to previous literatures, conceptual model was developed based on identified challenges of big data adaptation. To test whether these challenges essentially impact the big data usage, a quantitative analysis technique was used. To collect the data well-structured online questionnaire was distributed among 130 employees. Obtained information is analyzed through IBM SPSS software to find the correlation and regression. This analysis revealed that Sri Lankan authorities need to focus on infrastructure, trained workforce, security

standards, policies and education of employees in order to improve the big data usage. If required actions are not taken to improve the identified challenges, there will be a future trend of lack of big data usage in education sector of Sri Lanka. Moreover, there will be further consequences such as lack of big data usage for decision-making and impact profitability of education sector in Sri Lanka. Additionally, world is becoming globalized rapidly and there will be so much competition from other educational institutes in the world. Thus, big data usage would allow them to effectively understand and adopt to the market changes. Nonetheless, failure to use big data would cause most of the educational institutes in Sri Lanka to drive away from the international market. Finally, Sri Lankan economy is heavily depended on education sector for generating knowledgeable workforce that would contribute to the economy. If proper attention is not given by authorities to improve big data usage, the efficiency and productivity of education sector would hinder. Therefore, it is highly advisable that effective big data usage should be encouraged by overcoming the challenges identified in the research.

## *RECOMMENDATION*

Based on the findings and conclusion, infrastructure, training, policies, ICT education and security should be improved to encourage big data usage. Firstly, Infrastructure can be improved by use of software like Apache Hadoop which is a free open source software that could be used to store and process ample amount of data conveniently. Additionally, government should provide network facilities to all regional and rural areas. Government should also encourage local innovators by providing funding to develop analytic tools for data storing and processing. Moreover, government should develop a system where all educational institutes could purchase necessary infrastructure required for big data implementation at a lesser rate. Secondly, training of workers can be enhanced by providing a system where education sector employees could connect with industry professionals regularly to receive training about big data usage. Another way to increase big data usage is by training the amount of data scientist in the society. When amount of data scientist increases, other parties could obtain the guidance about the complex areas of big data usage. Thirdly, education institutes should enhance policies to encourage big data usage. Education system can change from manual systems to technological systems to record, store and analyze data. Technological systems would support big data usage. Next, Sri Lankan government should establish institute and regulatory frameworks to ensure the privacy and security of sensitive data due to the higher significance of big data utilization. Proper framework might gain trust among top managers of educational institutes to implement big data, mainly because they do not have to worry about the misuse of data and viruses that cause harm to sensitive data. Finally, education of employees needs to be enhanced to encourage use of big data. Government can incorporate computer science, statistics, and mathematics into Sri Lankan educational curriculum from ordinary level to university level. Moreover, implement strategic partnerships with private and public institutions with expertise in big data tools and techniques which allows to facilitate use of big data. Therefore, it could be highly understood necessary precautions and steps need to be taken earliest in order to encourage and motivate educational institutes in Sri Lanka to properly utilize big data in their daily operational activities.

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# STUDY ON WOMEN'S PERSPECTIVE TOWARDS AVIATION CAREERS IN SRI LANKA

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## *ABSTRACT*

The aviation sector is very important to Sri Lanka both in terms of contribution to employment as well as for the growth in Gross Domestic Product. The aviation sector is dominated by men rather than women. As according to the literature, one of the major issues that women have to face is lonesomeness while other factors including public opinions and political pressure. Besides, the women who were seek to enter for aviation related jobs have under gone challengers related to educational and occupational stereotypes in physical, cognitive and psychological abilities. It is imperative to understand the underrepresentation of women in other sectors in aviation other than in flight crew which induce professionalism to women in aviation. The purpose of this study was to identify the women's perspective towards aviation sector jobs in Sri Lanka. 10 in- depth semi structured face-to-face interviews were conducted with females who were selected as embedded - single case design method as per in statistics to order to represent different divisions in aviation. Prior to conduct in-depth semi structured face-to-face interviews, the questions were predetermined. 10 females from different divisions in a same organization was considered for the interview. The responders (10 women) were selected based on embedded - single case design and all the face-to-face interviews were conducted at the same day. Individual interviews were carried out to collect unbiased information from the responders. The research objectives were briefed to each interviewee prior to the interview. The snowball sampling method was enabled the interviewees to introduce the research for other responders who were in the target population. The results revels that there are several issues that women should overcome if they select aviation sector as their long term career path. In-flexible work schedules, lack of training opportunities, and also male work culture were identified as the major factors that influence of their work capacity. The gender imbalance can be rectified by implementing employer level and national policies which nurture more women in aviation. Majority of the women who are in the aviation sector jobs like to see more female representation in aviation sector jobs. Hence, as per this study reveals, it is mandatory to address the difficulties pertaining with in-flexible working hours for women in order to retain more women in aviation sector jobs.

Keywords: Aviation, Sri Lanka, Careers, Women

## *INTRODUCTION*

The aviation sector is very important to Sri Lanka both in terms of contribution to employment as well as for the growth in Gross Domestic Product. The aviation sector is dominated by men rather than women [1]. Hence, there have been very

low number of empirical studies carried out about women in aviation [2,6]. However, it is obvious that the majority of cabin crew and ticketing staff are women, while the behind the locked door of the cockpit, the situation is quite different [4]. By the change of social environmental situation occurred in 1970s, more feminine movement could be seen in aviation sector which were traditionally dominated by male [5]. As according to literature [3], one of the major issue a woman has to face is lonesomeness while other factors including public opinion and political pressure. Besides, the women who were seek to enter for aviation related jobs have under gone challengers namely educational and occupational stereotypes related to physical, cognitive and psychological abilities [6]. It is imperative to understand the underrepresentation of women in other sectors in aviation other than in flight crew which induce professionalism to women in aviation. The purpose of this study is to identify incentives and barriers which attract or limit women in general aviation sector in Sri Lanka.

## *METHODOLOGY*

Prior to conduct in-depth semi structured face-to-face interviews, the questions were predetermined. 10 females from different divisions in a same commercial airline based in Sri Lanka was considered for the interview. The responders (10 women) were selected based on embedded - single case design and all the face-to-face interviews were conducted at the same day. This approach produces healthy empirical qualitative data and it was allowed the respondents to discuss their opinions in explicit manner [7]. Individual interviews were carried out to gather unbiased information from the responders. The research objectives were briefed to each interviewee prior to the interview. The snowball sampling method was enabled the interviewees to introduce the research for other responders who were in the target population. The emails were sent for few members in the target population and these individuals were told to provide contact details of the other members in the same airline. Ultimately, it was ended up with 10 interviewees for the interview.

All the semi – structured interviews were carried out by a single interviewer in a day. A part from age, job profile and experience, 12 questions (Table I) were piloted with two aircraft technicians.

TABLE I. NTERVIEW SCHEDULE

TABLE I. INTERVIEW SCHEDULE

| Question No | Question |
|---|---|
| 1 | What are the factors which will improve the current working conditions? |
| 2 | What are the prominence issues which have to be deal with in the career? |
| 3 | What are the most important issues which affect for the working capacity, while working in the job? |
| 4 | How many training oppertunities did you complete before qualifying for your job? |
| 5 | Do you have opportunities to get promotions? |
| 6 | Do you have opportunities for novel developments? |
| 7 | Have you benefited from employer level policies which promotes gender equality in aviation sector? |
| 8 | Have you benefited from national level policies which promotes gender equality in aviation sector? |
| 9 | What are the causes for limited number of entries from women for jobs in aviation sector? |
| 10 | Do you motivate other women to work in aviation sector jobs? |
| 11 | Do you prefer to continue your current job as a long term career? |
| | **Open ended question** |
| 12 | What are any additional information on positive or negative work experiences which may be useful for this study? |

The interview process was started by inquiring the age, job profile and experience at the current job profile (Table II) from each responder.

TABLE II. LIST OF RESPONDENTS

| Respondent | From Introductory questions | | |
|---|---|---|---|
| | Age | Job profile | Experience (years) |
| 1 | 26-35 | Aircraft Technician | 6-10 |
| 2 | 26-35 | Ticketing | 0-5 |
| 3 | 15-25 | Management | 0-5 |
| 4 | 15-25 | Ground Handling | 0-5 |
| 5 | 26-35 | Information System Analysis | 0-5 |
| 6 | 15-25 | Aircraft Technician | 0-5 |
| 7 | 15-25 | Aircraft Technician | 0-5 |
| 8 | 15-25 | Management | 0-5 |
| 9 | 15-25 | Aircraft Technician | 0-5 |
| 10 | 26-35 | Ground Handling | 11-15 |

The rest other questions were related to identify the issues and challengers pertaining to limit women in aviation as given in Table I. Each interview was carried out 30 - 40 minutes. The responses were recorded separately. Prior to conduct this pilot study all participants noted that the questions did not make them to feel discomfort, hence they answered honestly. The interview was concluded with an open ended question which allowed the responders to give any additional information on positive or negative work experiences for women in aviation sector.

## *RESULTS AND DISCUSSION*

The 10 interviewees had an average age range of 15-25 and all of them have average range of experience from 0-5 years at the current designation. All the responders were females and out of that there were 2 females who are working as Aircraft Technicians, 2 as Ground Handling staff, 2 as Management staff and also 2 from Ticketing and 2 from Information System Analysis staff.

A. Responses related for factors which affect to improve the current working conditions

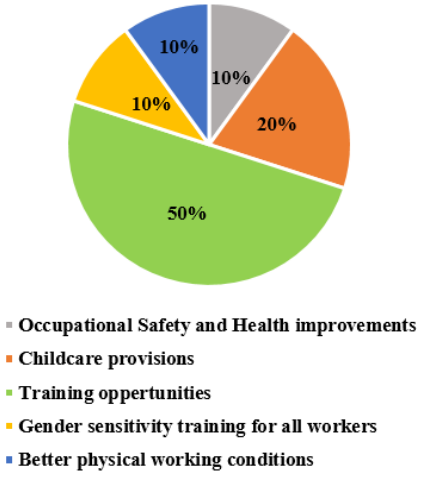


Fig. 1.Factors affect to improve the current working conditions

As shown in Fig. 1, half from the responders believe that providing the training oppertunities will improve the working conditions of the employees, while 10% from the responders believe that gender sensitivity training for all workers, better physicl working conditions and also improvements in occupational safety and health improvements will affect to enhance the present working conditions among female employees in aviation. Further, the rest 20% responded that Childcare provisions also influence to improve the current working condiitons.

B. Responses related for the prominence issues which have to be deal with in the career

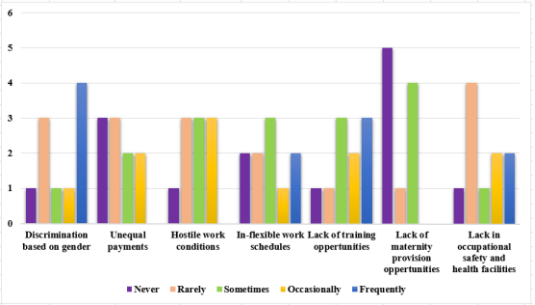

Fig. 2.Factors for the prominence issues which have to deal with in the career

As according to Fig. 2, seven parameters were identified which are directly relevant for the prominenet issues that the females under go during their career. As according to the responders only one mentioned that she has not gone through any sort of discrimination based on gender, while there are four females who have under gone discriminations due to femininity during their career. There were three females who experienced gender discrimination rarely during their career while other two females were experienced gender discrimination somethimes and occationally. Unequal payments were experienced rarely by three female emplyees, while another three never experienced such. Another two females were experinced unequal payments sometimes and another two by occasionally. But none of them experienced unequal payments frequently. As per the responders view there was one female who never experienced unfrienly working conditions while there were another three females for each who experienced hostile work conditions rarely, sometimes and occasionally. But there were non of the females who experienced unfriendly working conditions frequently. As per the information given by the responders, two of the responders have never under gone in-flexible work schedule while another two have frequently under gone through the in-flexible work schedules. There were three of the females who have experienced in-flexible work schedules sometimes. In addition there was one emplyee who experienced in-flexible work conditions occasionally while another two experienced in-flexible working conditions rarely. Three of the female responders mentioned that they have experienced lack of tarining oppertunities frequently while another three experienced lack of training oppertinities sometimes. One employee stated that she never experienced lack of training oppertinites and another one rarely experienced lack of training oppertunites. But two of the responders mentioned that they have experienced lack of training opperunities occasionally. Half of the respondents mentioned that they have never experienced lack in maternity provision oppertunities while another four respondents sometimes experienced lack in maternity provision oppertinities. One has expeirenced lack in maternity provisions oppertunities rarely but none of them experience lack in maternity provision oppertunities frequently. Four of the respondents have rarely under gone lack in occupational safety and health facilities while another one never experienced lack in occuptonal safety and health facilities. Another two females experienced lack in occuptonal safety and health facilities occasionally and other two experienced it frequenty. In addition there was a single respondent who experienced lack in occuptonal safety and health facilities sometimes.

C. Responses related for the most important issues which affect for the working capacity, while doing in the job

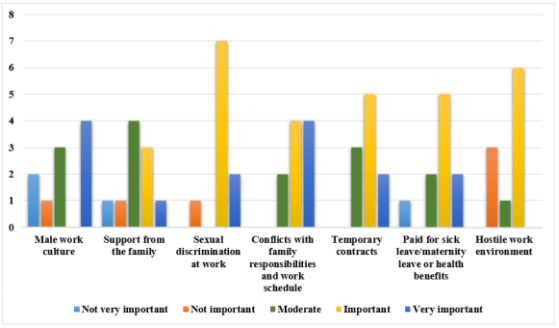


Fig. 3.Factors which affect for the working capacity

As given in Fig. 3, six factors were identified which have the influence on the working capacity of female employees. Four respondents mentioned that the male

work culture is very important and two females mentioned that male working condition is not very important of their working capacity. Three of the employees mentioned that male work culture is moderately influence for females working capacity while none of them mentioned it as important factor of their working capacity. But there was one respondent who mentioned that male work culture is not imporatnt for her working capapcity. Four of the respondents mentioned that support from the family moderatly influences for the working capacity and another three mentioned that support from the family effect for the working capacity importantly. One of the respondents mentioned that family support is not very important for the working capacity for her while each one mentioned that support from the family is not important for the working capacity and the remaining one stated that support from the family is a very important factor for her working capacity. Seven of the responders stated that the sexual discrimination at working place importantly influence for the working capacity and two of the respondents mentioned that sexual discrimination is a very importantly influence for the working capacity. Only one respondent mentioned that sexual discrimination at the working place is not important for the working capacity of her. Four of the responders mentioned that conflicts with family responsibilities and work schedule moderately influence for the working capacity while another four stated that conflicts with family responsibilities and work schedule very importantly influence on their working capacity. The rest of the responders mentioned that conflicts with family responsibilities and work schedule moderately affect for their working capacity. Five from the total respondents mentioned that by being in temporary contract in job importantly influence for the working capacity while three of the responders stated that being in temporary contracts moderately influence for the woking capacity. The rest of the responders stated that being in temporary contract plays a very important role in working capacity of them. Five respondents mentioned that paid for sick/ maternity leave or health benefits are important for working capacity while two respondents mentioned that paid for sick/ maternity leave or health benefits are very important for working capacity. Another two respondents stated that paid for sick/ maternity leave or health benefits are moderately important for the working capacity while one respondent mentioned that paid for sick/ maternity leave or health benefits are not important for her working capacity. Six respondents mentioned that hostile work environment is importantly influence for the working capacity and one mentioned that hostile work environment is moderately influence on working capacity for her. The rest of the respondents mentioned that hostile work environment is not very important factor to influence their working capacity.

D. Responses related for training oppertunities before qualifying for the specific job

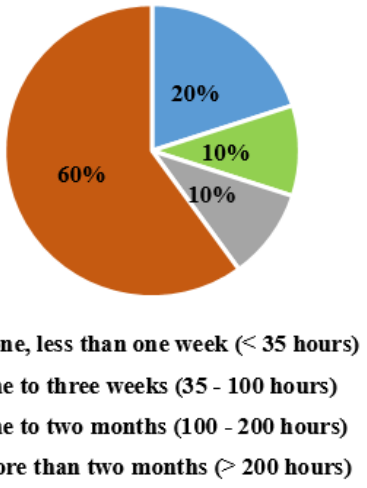

Fig. 4.Training oppertunities prior to job

As shown in Fig. 4, 60 % from the respondents have gone through a training for more than two months before they start the specific job and 20 % of the females have gone through none or less than one week training programme. There were 10 % from the respondents for each who have done the training for one to three weeks and one to two months respectively.

E. Responses related for the oppertunities for promotions

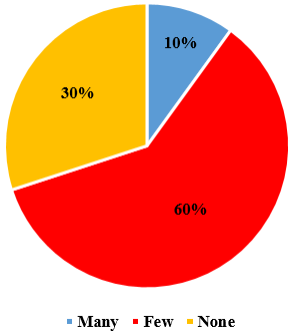


Fig. 5.Oppertunities for promotion

As according to Fig. 5, 60 % from the respondents have obatined few oppertunities for promtion while another 30 % from the respondents have obatined none of the oppertunities for promotion. The rest of the respondents obatined many oppertunities for promotion.

F. Responses related for oppertunities for new developments

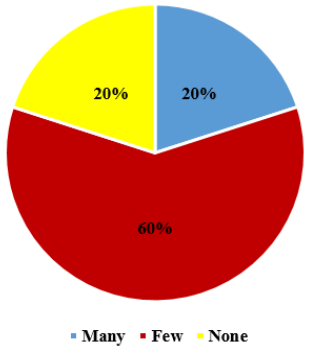


Fig. 6.Oppertunities for new developments

As shown in Fig. 6, 60 % from the respondents have got opportunities to do new development while 20 % have received many opportunities for new developments. The rest of the females never got an opportunity to do novel developments during their career so far.

G. Responses related for the impact of employer level policies which nurture women in aviation

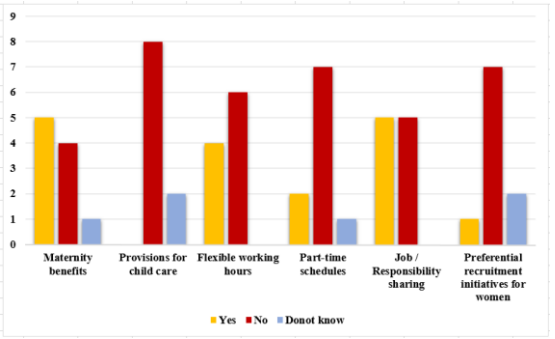


Fig. 7.Impact of employer level policies to promote women in aviation

As Fig. 7 illustrate the identified factors based on employer level policies which affect to nurture gender equality. Half of the respondents mentioned that providing maternity benefits has an influence on promoting female in aviation, while four respondent mentioned that they do not think that providing maternity benefits nurture women in aviation sector. In addition, one respondent stated that she did not have any idea about it. 8 of the respondents mentioned that provisions for child care do not have any influence on promoting women in aviation while the rest stated that they do not know about it. Six of the respondents mentioned that by providing only flexible working hours for ladies may not influence women in this sector and four of the females stated that providing flexible working hours may influence women in aviation sector jobs. Seven of the respondents mentioned that

by providing part-time schedules will not nurture women in aviation and one responded against to it. Two respondents mentioned that by providing part-time schedules will lead to nurture women in aviation. 50 % of the respondents agreed that the feasibility of sharing responsibilities may nurture women in aviation while the rest of the respondents disagreed with it. Seven of the respondents mentioned by providing only preferential recruitment initiatives for women will not influence women in aviation while one was against to it. Two of the respondents mentioned that they did not have any idea about it.

H. Responses related for the impact of national level policies which nurture women in aviation

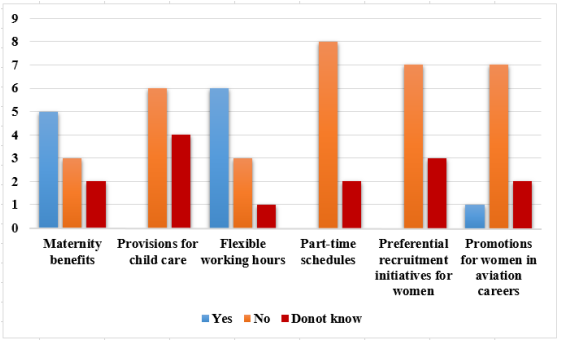


Fig. 8.Impact of national level policies to promote women in aviation

Based on the respondent's comments six factors were identified which have (Fig.8) the impact to nurture women in aviation in national level. Half of the respondents mentioned that national level policies for maternity level benefits may encourage to nurture women in aviation sector and three of them were not agreed with it, while the rest of the respondents mentioned that they did not have any idea about it. Six of the respondents stated that national policies of provisions for child care will not nurture women in aviation while the rest was against to it. Six respondents mentioned that national policies on flexible working hours will influence to have more females in aviation sector and three were disagree to it. But one of the respondents mentioned that she did not think that national level policies on flexible working hours may effect to enhance the number of women in aviation. Eight of the respondents mentioned that national level policies on part-time schedules will not nurture women in aviation while two respondents did not have any idea about it. Similar responses were obtained for the preferential recruitment initiatives for women as well, where seven respondents were agreeing with it and the rest was against to it. Seven respondents stated that national level policies on promotions will not nurture women in aviation while one was agreeing with the statement. There were two respondents who did not know about it.

I. Responses related for limited number of entries from women for jobs in aviation sector

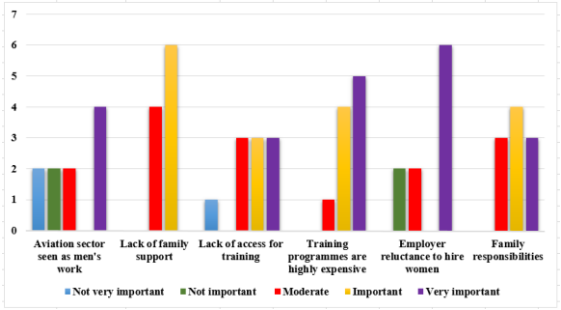


Fig. 9.Factors related for the limited number of women entries in aviation sector

Based on the respondent's comments six factors were identified (Fig 9) which influenced for the limited entries of women in aviation sector. Four respondents mentioned that very importantly they seek aviation as men's work sector while two were totally against to it. In addition, another two females moderately agree with the statement while

another two did not think importantly that the aviation sector is only for men. Six from the respondents seen that lack of family support is one of the important factor for not choosing aviation sector jobs by women. The rest of the women had moderate consent on it. Three of the respondents mentioned that lack of access for training opportunities was very important factor which limit women entries in aviation sector jobs and three respondents were moderately agreed to it. But one respondent stated that lack of access for training was not very important factor for limited women in aviation sector jobs while the three of the respondents considered it as an important factor. Six of the respondents mentioned that employers are reluctance to hire women in aviation sector and as per them it was very important factor which limit women entries in aviation but three of the respondents did not think it as an important factor while the rest of the respondents think it as a moderate factor for women entries in aviation. Three respondents mentioned that family responsibilities play a very important role which lead to limits women entries in aviation and another three females were moderately agree to it, while the rest of the responders were considered it as an important factor which restrict women entries in aviation sector jobs.

J. Responses related for promoting aviation sector jobs among other women

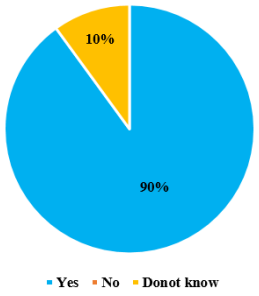


Fig. 10. Promoting aviation jobs among other women

As shown in Fig. 10, 90 % of the respondents have the consent to promote aviation sector jobs among other women while 10 % of them have no idea yet.

J. Responses related for aviation sector jobs as a long term career

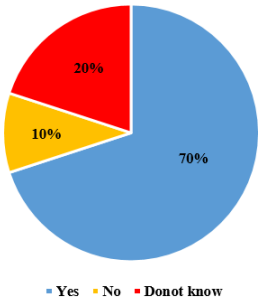


Fig. 11. Selection of aviation as a long term career

As given in Fig. 11, 70 % from the respondents stated that they select their present career as their long term career while 10% mentioned that their current job isnot their long term career. 20 % of the respondents mentioned that they have not decided yet whether their current job will be their long term career or not.

K. Responses related for any additional information on positive or negative work experiences for women in aviation sector

TABLE III. GENERAL COMMENTS BY RESPONDENTS

| Respondent | Responses for the additional information related to this study |
|---|---|
| 1 | Family support is very important as a lady in working in the aviation industry. In addition, Child care support facilities will definitely add more value to a working mom. |
| 3 | Transport is a main issue that will demotivate the women. |
| 8 | Transport is an issue. So females have to boarded close by. Hence this leads to arise family conflicts. |

Table III consist with the responses which were given by the respondents for the open ended question which was raised by the interviewer. 1st respondent mentioned that family support plays a major role for a woman who occupy in the aviation sector. Besides she mentioned that providing child care support facility is very important for a female who is working in the aviation sector. 3rd and 8th respondent mentioned that transport is one of the issues to demotivate ladies in the aviation sector. Further, 8th respondent stated that when they boarded away from their home, it leads to miss some of the day today responsibilities. It may lead to arise family conflicts as well.

## *CONCLUSIONS*

It is evident that women who are in the aviation sector still believe that they have to overcome several hurdles than their peer males in the aviation sector. Majority of the respondents suggested that the current working conditions can be uplift by providing more training opportunities and also by providing more childcare provisions. The respondents mentioned that they are frequently undergone issues such as discrimination based on gender, in-flexible work schedules, lack of training opportunities and also lack in occupational safety and health facilities. Similar factors were identified by the literature [2, 8], as attributes which women concern when they select aviation sector as their career path. Majority of the respondents agreed that male work culture at the working place and conflicts with family responsibilities are major issues which effect for their working capacity. 60 % of the respondents have gone through training prior to their job. 60 % of the respondents got few opportunities to do new developments in their working place and also 60 % of the respondents got few opportunities to promote. As per the responses employer level and national level policies are very important to nurture women in the aviation sector. This is agreeing with the literature [6], which mentioned that policies and regulations should be brought to implement gender equality in aviation sector. In this study majority of the respondents seen aviation as men's work which is contradictory with literature [8]. There were several factors were also identified which limit women entries to aviation such as lack of access for training, training programs are highly expensive and also employers are reluctant to recruit women for aviation jobs. In addition, higher family responsibilities in motherhood also limit more women entries in aviation, which is also coincide with the literature given in [1]. Most of the respondents like to promote aviation sector jobs among other women which is also similar with the research findings in literature [8]. Further, this study reveals that 70 % from the respondent would like to continue their present aviation sector job as their long term career, but they noted that they should able to balance their family life as well as career life due to being women, they have more responsibilities towards childcare and family life than men. Further in-depth research needs to be done with more number of respondents in order to generalize these findings through the populations.

**Acknowledgment**

The author would like to thank for all the interviewees for sharing their time and experiences with us to carry out this study successfully. Further, the author would like to express many special thanks to Mr. S. Jayasiri his assistance in this research.

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# PHARMACOGNACY OF PATHTHU DRAVYA FOR AMAVATHA SHOTHA USED BY NADUNGAMUWA WEDA PARAMPARA

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## *ABSTRACT*

Chronic/Acute inflammatory joint disease Amavata (Rheumatoid Arthritis) is a disease that some medical systems are failed with treating. This causes swelling, pain and stiffness of joints.Asayurveda it manifested in joints of hasta, pada, sira, trika, gulpha, janu and uru. The main symptoms produced are Angamarda Aruchi, Trishna, Gouravam, Apaka & Shotha.Abovepaththu is most significantly used and Specific objective of this study was to study the pharmacognostic aspect of its ingredient herbs and other pathu herbs used by this tradition for shotha. As the Ingredients of paththu Thala, Sathakuppa, Eranda seeds, Aralu, Rathhadun, and Siyabalaleaves, prominently it shows Thiktha-Madhura rasa reading the Rasa property. And as to the descending order of other rasa katu-Kashaya-Amla rasa can be seen. Lawana rasa was not found. According to the Guna Property mostly Lagu-Ruksh-Thikshna guna were included and snigdha-Sara-Guru guna were containing in very less amount. Regarding the Property of Veerya.It contains 78% of Ushna Veerya and 22% of Sheetha Veerya. Under the property of Vipaka 80% of drugs in Katu Vipaka , 20% madhura vipaka and 4% in Amla Vipaka.So the Amla Vipaka herbs used in very little. Therefore theshotha contributed by Madura Rasa Vipaka, Ushna Veerya, and Vatha dosha willbe removed and thiktha Rasa, ushana veerya and kapha dosha became to shaman state. It clearly shows in the traditional ama vatha shotha paththu reduce the shotha condition by contributing shamana of Vata and kappa dosha very well.

Keywords Shothahara, Amavatha, Paththu, Dravya, Guna

## *INTRODUCTION*

Sri Lanka is a country of rich heritage, one of which is its indigenous system of Medicine, which has been practiced by the people since time immemorial. Traditional medicine is the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. Most of the indigenous medical practitioners in the island were treating many patients daily. Maximum number of patients was attended by trained practitioners. A traditional healer can be defined as a person who is recognized by his/her community as competent enough to provide healthcare by using herbs, animal and mineral substances, or other methods. These methods are based on social, cultural and religious principles, including knowledge, attitudes and beliefs regarding the physical, mental and social well-being that are prevalent in their community.

Literature Review

Categories of Sri Lankan Traditional healing system:

1. Sarwanga Roga
2. Sarpa Visha
3. Lama Roga
4. Kedum Bidum
5. Dewum Pilissm
6. Charma Roga
7. Nila Weda kama
8. Gedi Wana
9. As Wedakama
10. Visha Wedakama
11. Garbhani Roga
12. Yantra Mantra
13. Kem Krama

In these traditional systems, as the disease condition different adjuvant (anupana) or sahapana was used. According to the Vatika Prakaranaya there were many examples of remedies with anupana, guli Kalka especially for sanni, kola, suthika, upadrawa conditions which may need antibiotic effects as modern treatments. That will illustrate the power of these special drug preparations in this healing system. Not only that this system may contains physiology, anatomy, pharmacology, unique measuring system, treatment procedures, and indication and contra indications as a complete medical system.

Unique characteristics of the srilankan traditional healing system:

- It is an important fact in srilankan traditional haling system is “weda geta”. It was a creation of formularies to each disease .That was included with in a poem as a puzzle. That can be only use for the peoples who have sound knowdge on traditional healing systems.

Example: Abhu - Kaladuru
Wada Genige Kes - Wadha kaha
Mrutha - Pawatta Kollo
- Kollan Kola
Badha Helayan - Rankubala
Katu Athi Gasa - Andara
Goyiyo - Goyi Wel
Naketh Kiyana Aya - Niwithi
Kumbure Madha - Kumburu Ata
Madhagedi Athi Gasa - Rata Edaru
Hema Rasa Phala - Nelli

By making this kind of recopies and their indications were kept with safe for

1. Avoid Knowing these knowledge by outsiders of each tradition
2. To memorise easily
3. And to have some pleasure

- Anatomical Knowledge Paired to ayurveda different just for a wards only.

Example: - Shroniya - Ukula - hip
Gulpha Sandi - Bolataya - ankle
Parshu - Ila ata - ribs

- Special unique disease nomenclature.

Comparing to ayurveda there is high number of disease as physiology

Example:- Mandham Roga–20 Pinas Roga -18

- New treatment modalities

Example: - Enchanters (Yantra Mantra), Padmana (use of powder drugs), EPA nul bedima, Pillissum, Bhagna, Nila Vidima, Anpana. Oil, well Kashaya, Ala beth, Choorna Thawili, Paththu, Wedu.

- Measurements(Mana Paribasha)
  Ex: - Madatiya eta, Olinda Eta, Wee Eta, Amu eta } used as a dosage quantity

History and importance of the traditional gedi wana and sarwanga chikissa

As the Gedi wana tradition, Gedi is the conditions where harmful external entities are enter to the humenbody and caused swelling, inflammation, spuration, pus discharge to remove those foreign matters by skin. This condition known as gedi and vidradi, arbudha, Pidaka are varities of gedi.Statement of traditional doctor about Gedi: - it is an abnormal swelling of the body. It can be appear any part of the body and affect you are health condition. Some of them and can be cure by the proper treatment. Statement of traditional doctor about Wana it is a wound can appear any part of the body. Some wound care can cure easily but some are difficult to cure and also cause major problem to the health. According to the gedi wana weda potha there are 400 gedi. As sarvawishadi herbal oil recepy 4800 number of gedi were mentioned. Beth thel potha mentioned 48 gedi and they named as each areas. Gediwana are describing in many type according to the shape and place.as an examples Gadapola, Visara, Gedi, Leh gedi, Vissappu etc.

01.Hubas bandana
02.Nalalgadawa
03.Bemapilike
04.Nuvanpilika
05.Nahadanu pilika
06.Danthapilika
07.Hakupilika
08.Iranthugadava
09.Kandamala leda
10.Kanaka visadi
11.Urabandana
13. Kihilibandana
14. Rathapulliya
15. Ath pilika pa pilika
16. Deva amaka
17.Kondara pilka
18.Odduvisahi
19.Upadansa pilika
20.Kondarapilika
22.Deepangar darvaya
23.Upadansa pilika
24. Galrathaya
25.Thunbitu bandanaya
26. Maspilikegauwa
27Pokuru visadi
28.Sandi bandanayas
29.Sanni musappu
30.Kalal bandana
31.Udarabandana
32 Dewasura amaka
33.Katupilika
34.Ilapala gadava
35.Elabandana
36.Kondaragauwa
37.Lemapilika
39.Kikili kukula
40.Sanni bandana
42.Alabandana

In traditional Healing System The Gedi condition Introduced by Poem.This knowledge to ayurveda students. Dr. P.K.H.Dharmavijaya used his great knowledge as well as good treatment procedures, Nila chikissa and he also conserning the mental states of patients specially.

**Nadungamuwa veda parampara**

Figure1:Dr.P.K.H.Dharmavijaya

**Nadungamuwa veda tradition**

Dr.P.K.Harsha Dharmavijaya was the representater of this tradition in this era and my teacher belongs this elephant from his father Dr.P.C.G. Dharmavijaya in the year of 1978.Now the nedungamuwa raja was the identity of nedungamuwa weda parampara.This tradition contains long written history on gedi wana, pidaka, and sarwanaga treatments.And still surviving in gampaha district, Rathupaswala, nedungamuwa village. It's originated in mathale district and then to dekatana and finally belongs to gampaha district. The originater of this tradition was Dr.livinis perera paduwawala,from him to dr.basthiyan perera paduwawala and then to Dr.P.D.G Dharmavijaya (paduwawala kankanamge dhaniyel perera dharmavijaya weda rala hami) he practiced this treatments well and he was a(D.A.M.S) abhighana pathara dhari of Ashtanaga ayurveda collage of kalkata in india . Not only had that he maintained a simple religious life style too. It is divided into 4 generations. Present Dr.P.K.Harsha Darmavijaya who has taken the knowledge from yakkla siddhayurveda vaidhya vidhyala and with his traditional knowledge treating for many patients very well

**Important Concepts in Nedungamuwa Traditional Healing Tradition**

1. Consider seasonal effects-ushna sheetha cosept in foods and medicineshigh usha quality - "surya" Ghana aushada High sheetha protency - "chandra" Ghana aushada

2. Pharmacognacy -There are different formularies as each tradition

Andover medicinal formularies were called as water. Each water was formulated considering Rasa, Guna, Virya, Vipaka etc.

3. Pathya Apathya- Acording to the treatment type patients should be followed special maintenance with their food habit and behaviors in shoran karma bathing cold water having cold food prohibited.

Literature review of medicinal plants used as the ingredients

Scientific name: Terminally chebula

Family name: COMBRETACEAE

English name: Chebulic Myrobalan

Local name: Aralu (අරලු)

Sanskrit name: Harithaki / Abhaya

Description: Trees to 30 m tall; branchlets conspicuously white or yellowish long lenticellate, glabrous, hairs tawny, rarely silvery. Leaves alternate or sub opposite, spaced along branch lets; petiole 1-3 cm long, with 2(-4) glands 1-5 mm below apex; leaf blade 7-18 × 4.5-10 cm, glabrous. Inflorescences maxillary or terminal, simple spikes, 5-10 cm long, numerous flowered, sometimes grouped at branch let apex and forming a panicle. Flowers slightly fragrant, bisexual. Fruit not stipulate , blackish brown when ripe , ovoid or broadly so, ellipsoid , or cylindrical-ovoid, obtusely 5-ridged, 2-4.5 × 1.2-2.5 cm, rigid , becoming deeply wrin.

*Figure1: seed-flower and unripe fruit of terminality chebula*

Edible parts: Seed/ leaf/ Cotyledon

Ayurvedic usage: Treatment for Dentalcaries,Bleeding gums ,Fevers, Eye diseases, Piles, Dropsy, Sores, Chronic dysentery, Worm infestation, Swellings, Hemorrhoid, Jaundice,Colds,Coughs, Catarrh, Anorexia, Flatulence, Abdominal discomfort, Eczemas

Parts used in treatment: Pericarp of the fruit

Related medicinal properties: Cardio tonic, Purgative, Stimulate liver functions, Diuretic, Pacifies vitiated thridoshas

Scientific name: Pterocarpus santalinus

Family name: FABACEAE

English name: Red sandal wood, Red sanders, Ruby wood

Local name: Rath handun

Sanskrit name: Raktha chandana

Description: Tree to about 30 m tall; stems glabrous. Leaves 4-8 foliate; leaflets 5.5- 12(-15) cm long, 2.5-7 cm wide, glabrous. Inflorescences paniculate. Flowers 12-19; petals bright yellow, glabrous. Fruit 1 or 2 seeded, about 4-5.5 cm in diameter, the marginal wing1-2 cm wide; seeds brown 10-12 mm long, 5-6 mm wide.

*Figure2: leaves and bark of Pterocarpus santalinus*

Status: Only under cultivation

Edible parts: Bark

Ayurvedic usage: Treatment for- Swellings, Vomiting, Boils, Inflammations, Eye diseases, Excessive thirst, Headaches, Dysentery, Fevers, Hemorrhoids, Burning sensations of the body, Skin diseases, Worm diseases

Parts used in treatment: Heartwood

Related medicinal properties: Purifies the blood, cooling agent,

Pacifies vitiated Kappa and Pita Doshas

Scientific name: Anethum graveolens

Family name: APIACEAE / UMBELLIFERAE

English name: Dill seeds

Local name: Sathapushpa

Sanskrit name: Shathavah, shathapushpa

Botanical Description: anthem graveolens L. Is the sole species of the genus Anethum, though classified by some botanists in the related genus Peucedanum as Peucedanum graveolens variant called East Indian dill or Sowa (Anethum graveoeloens var sowa Roxb. Ex, Flem.)


*Figure3: seeds leaves and flowers of anethum graveolens*

Description: Glabrous, branched, perennial herbs with striated green stems covered with a whitish bloom. Leaves alternate, decompounds; ultimate segments linear-oblong or ovate, entire or sparingly toothed. Umbels compound, rays usually numerous; bracts linear or linear-lance late; bracteolate 4-8, linear. Flowers with many slender pedicels. Sepals 0 or small. Petals obovate, emarginated or 2-fid. Ovary glabrous. Style small. Fruit 2-3 times as broad as thick, vittate large.

Status: Naturalized Exotic

Edible parts: Seed

Photochemical: plant showed the leaves, stems and roots were rich in tannins, terpenoids, cardiac glycosides and flavonoids

Ayurvedic usage: Treatment for- Dysentery, Diarrhea, Catarrh, Stranger, Cystitis, Urinary bladder disorders, Hemorrhoids, Otorrhoea, Burns, Constipation, Ulcers, Wounds, Gonorrhoea,Coughs,Head lice, General pains, Nervous system disorders.Rasa-Katu thiktha Guna-lahu Thikshna Vipaka – katu Veerya-Ushna

Parts used in treatment: Leaves, Seeds

Related medicinal properties: Aphrodisiac, Lactogogue, Diuretic, Vermifuge, Purgative Pacifies vitiated Catha Doshas and Balance vatha Kapha Increase pitta

Scientific name: Ricinus communis*

Synonyms: Rakta Erandashwetha Eranda

Family name: EUPHORBIACEAE

English name: Castor

Local name: Eradu

Sanskrit name: Eranda

Description: Avery variable plant in habit, and appearance; annual or perennial, 2.5-3.5 m tall; stems hollow, cylindrical. Leaves simple on curved, purplish or green petioles, subpeltate drooping; stipules large, green or yellowish; lamina 15-45 cm across, palmately cut into 7-11 serrate blue-green segments. Inflorescences spicate panicles. Flowers monoecious, large, Fruit a blunt, greenish, deeply grooved, tricoccus capsule, about 2 cm long and septic dally into 6 valves. Seeds ovoid, 0.8-1.2 cm long, 0.6 cm broad, prettily mottled with dark brown, albuminous.

*Figure4: Fruit seed leaves and flowers of Ricinus communis*

Pharmacological: presence of Steroids, Saponins, Alkaloids, Flavonoids, and glycosides

Status: Naturalized Exotic

Edible parts: none

Ayurvedic usage: Treatment for- Rheumatoid arthritis, Osteo arthritis, Chest pains, Oedema,Fevers, Nervous system diseases,Pains,Bleeding gums,Eczema,Constipation,Worm diseases, Hemorrhoids, Splenomegaly, Hydrocele,Coughs,Oede,Ear ache

Parts used in treatment: Roots, Bark, Leaves, Seeds

Related medicinal properties: Purgative, Stimulate lactation, Anthelmintic

Parts used in treatment: Roots, Bark, Leaves, Seeds

Related medicinal properties: Purgative, Stimulate lactation, Anthelmintic

Scientific name: Tamarinds indicia*

Family name: FABACEAE

English name: Tamarind

Local name: Siyambala

Sanskrit name: Thinthidi

Description: Trees to about 15(-24) m tall; stipules minute. Leaves with about 8-20 pairs of leaflets; leaflets 12-(-30) mm long, (3- )5-10 mm wide. Inflorescences racemose, about 1-8 flowered; flowers small, pedicels 3-14 mm long; petals white or yellowish with reddish venation. Fruit light brown, 5-15 cm long, about 2 cm wide, 1.5 cm thick, 2-4-seeded, indehiscent.

*Figure5: leaves flowers fruits of Tamarindus indica*

Edible parts: Fruit, Bark, leaves

Ayurvedicusage:Treatment for; Swelling, Pain, Excessive thirst, Burning Sensation, Fever Hemorrhoids, Oral Diseases, Rectal Prolepses, Constipation,Dysuria, Leucorrhoea,Vomitting,Eye Disease, Muscle stiffness, Hernia

Pharmacological: Rasa-Madhura, Katu, Kashaya Guna- Guru, snigdha, tikshna, suksma Veerya-Ushna Vipaka-Madhura Dosa-Kaphavata shamaka, Karma-Sulahara, shothara, rechana, brushy, twachya, vedanasthapan, kriminihsarak, hridyashoth, kaphaghna, mutravishodhna, stanyajanana, sukrashodhana, kusthaghna, jwarahara, svedajanana, balya, garbhashayashodhan, visaghna, vayahsthapan

Parts used in treatment: Leaves, Bark, Flowers, Fruits, Seeds

Scientific name: Datura metel
Family name: SOLANACEAE
English name: Adams apple
Local name: Attana (අත්තන) / Kalu-attana (කළු-අත්තන)
Sanskrit name: Dhattura, Dhastura, Unmatta, Shivapriya, Harapriya, Hema, Dhustura, Kanaka

Description: Erect, rounded, soft stemmed shrub, up to 1(-2) m tall. Leaves entire or angular in outline, up to 29 cm long, 16 cm broad; petioles 1/3-1/2 as long as blade. Flowers erect solitary in axils; corolla about 15 cm long, creamy white or streaked with red or mauve. Fruit a spherical capsule covered with stout tubercles or prickles. Seeds brown, 5 mm diameter.

*Figure7: Flowers Leaves fruit and Seeds of dater metal*

Photochemical: Saponins, tannins, steroids, alkaloids, polyphenols and glycosides in this plant Rasa Tikta, Katu, Guna Laghu, Ruksha, Vyavayi, vikasivirya ushnavipaka-Katu. Prabhava,-madakadoshaghnata Kaphavatashamaka.Rogaghnata-Shotha, Vedana, Arsha, Vatavikara,Hridmandata, Nadimandata, Amlapitta,Parinamashoola, Pittashmari, Shwasa, Vrikkashoola, Ashmari, Shaiyyamootra ,Rajahkrichchhra, Yuka, Liksha Karma-Jantughna, Vedana sthapana,Twagd oshahara, Madaka, Antrashamaka, Shoola prashamana, Hridayottejaka, Bastiavum Gavinisankochaka, Garbhashay aprasaraka, Shukrastambhana, Swedavarodhaka. Contains different functional groups such as saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides. Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants. Saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides. Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants.

Status: Native

Edible parts: Leaves, Seed, Root, seed oil, flower, panchanga

Ayurvedic usage: Swellings,Rheumatism,Lumbago,Tumours,Cataract,Eye diseases,Asthma,Toothache,Dogbites,Hydrophobia,Hydrocele,Fevere,colds,Tuberculosis,Insanity,Abscesses,Sciatica,Dysmenorrhoea,Generapains,Rabies, Filariasis.Antispasmodic, Anodyne,Narcotic

Parts used in treatment: Leaves, Seeds, Root.

## *METHODOLOGY*

This study therefore was carried out to investigate the pharmacognacy of ingredients in apaththu used by nedungamauwa weda parampara for ama vatha and its effacy also observed during the period of 3 months.Dr.P.K.Harsha Dharmavijaya was the representater of this tradition in this eraget the training under his guidance for 3 months. Different types of paththu varga was collected and observed their usage. Data was collected by the sanhitha grantha and other subject related records and the ingredients of paththu dravya were analyzed with their pharmacognacy.

**Preparation of methods of some paththu varieties**


*Figure8: Preparation of Shothahara Paththuwa*

**Preparation of Delipothu paththu**


*Figure9: Preparation of delipothu paththu*

## *DATA ANALYSIS*

Study of Rasa Guna Veerya Vipaka

| Name | Rasa | Guna | Veerya | Vipaka | Dosha Karma |
|---|---|---|---|---|---|
| ***Deli pothu*** | | | | | |
| ***Thiththa abin*** | *Katu,Thiktha* | *Lagu -Ruksha* | *Ushna* | *Katu* | - |
| ***Viyan dubulu*** | *Katu,Thiktha,K ashaya* | *Lagu -Ruksha* | *Ushna* | *Katu* | - |
| ***Perumkayan*** | *Katu* | *Lagu-snigda-Sara-Thikshana* | *Ushan* | *Katu* | *Pitta Wardaka, Kapha vatha shamaka* |
| ***Aththana Leaves*** | *Madhura,Katu, Thikth, Kashaya* | *Guru,Thikshna* | *Ushna* | *Katu* | - |
| ***Nika Leaves*** | *Katu,Thiktha,K ashaya* | *Lagu -Ruksha* | *Ushna* | *Katu* | *Kapha Vata Hara* |
| ***Wara Leaves*** | *Katu-Thiktha* | *Lagu-Ruksha-Sara-Thikshana* | *Ushna* | *Katu* | *Kapha Piththa Shamaka* |
| ***Thana hal*** | *Madura-Kashaya* | *Sheetha-Mrudu* | - | - | = |
| ***Cow's milk*** | *Madhura-Amla* | *Guru-snigda* | *Sheetha* | *Madura* | *Kapha wardaka, Piththa shamaka* |
| ***Thala*** | *Madura* | *Guru-Mrudu-Snigda* | *Ushna* | *Madhura* - | *Increases pitta, Decreases vāta* - |
| ***Sathakuppa*** | *Katu thiktha* | *Lahu Thikshna* | *Ushna* | *Katu* | *Pacify Vata* |
| ***Eranda seeds*** | *Madura-Katu-kashaya* | *Snigda-Sukshma-Thikshna* | *Ushana* | *Madura* | *Kapha vata Shamaka* |
| ***Aralu*** | *Pancha rasa* | *Lagu-Ruksha* | *Ushan* | *Madura* | *Tridosha Shamaka* |
| ***Raththadun*** | *Madura-Kashaya* | *Lagu-Ruksha* | *Ushana* | *Katu* | *Kapha Piththa Shamaka* |
| ***Siyabala leaves*** | *Amla* | *Amla-Guru* | *Ushna* | *Katu* | *Kapha vata Shamaka* |
| ***Murunga*** | *Katu-Thiktha* | *Lagu-Ruksha-Thikshna-Sara* | *Ushna* | *Katu* | *Pitta wardaka,Kapa vata* |
| ***Aba*** | *Katu-Thiktha* | *Ruksha-Thikshna-Snigda* | *Ushna* | *Katu* | *Pitta wardaka, Kapha vata Shamaka* |
| ***Sarana Mul*** | *Madura-Kashaya-Thiktha* | *Lagu-Ruksha* | *Ushna* | *Madura* | *Kapa Vata Shamaka* |
| ***Dewadara*** | *Katu-Thiktha* | *Lagu-Snigda* | *Ushana* | *Katu* | *Kapa Vata Shamaka* |
| ***Amu Inguru*** | *Katu* | *Guru-Ruksha-Thikshna* | *Sheetha* | *Madura* | *Kappa kappa vatha shamaka* |
| ***Sudulunu*** | *Katu- Madura* | *Guru- snigda-Thikshna-sara pichchila* | *Ushna* | *Katu* | *Kapa Vata Hara* |
| ***Meepothu*** | *Madura-Kashaya* | *Snigda* | *Shitha* | *Madura* | *Vatha Pitha Shamaka* |
| ***Harankaha*** | *Katu -Thiktha* | *Lagu-Thikshna* | *Ushna* | *Katu* | *Kapa Vata Shamaka* |
| ***Lunuwarana pothu*** | *Madura – Thiktha-Kashaya* | *Lagu-Rksha* | *Ushna* | *Katu* | *Kapa Vatha shamaka* |
| ***Iramusu*** | *Madura-thiktha* | *Guru-snigda* | *Shitha* | *Madura* | *Tridosha Shamaka* |
| ***Kapuru*** | *Madura-Katu thiktha* | *Guru- Thikshna* | *Ushna* | *Katu* | - |
| ***Hiressapalu*** | *Madura-kashaya* | *Lagu-Ruksha* | *Ushana* | *Amla* | *Kapa Vatha Shamaka* |
| ***Amukaha*** | *Katu- Thiktha* | *Lagu-Ruksha* | *Ushna* | *Katu* | *Pittha Virechaka,Kapa Vatha Shamaka* |
| ***Suwadakottan*** | *Madura-Katu-Thiktha* | *Lagu-Ruksha* | *Ushana* | *Katu* | - |
| ***Kapu eta*** | *Madura-Kashaya* | *Lagu-snigda* | *Ushna* | *Madura* | *Vatha Shamaka,Kapa piththa Wardaka* |
| ***Katuwelbatu*** | *Katu- thiktha* | *Lagu-Thikshna-Ruksha* | *Ushna* | *Katu* | *Kapa Vatha Shamaka* |

| | | | | | |
|---|---|---|---|---|---|
| **Kaburu eta** | Katu- thiktha | Lagu- Ruksha | Ushna | Katu | Kapa Vata Shamaka |
| **Kidaran** | Katu- Kashaya | Lagu- ruksha | Ushna | Katu | - |
| **Rathnitul** | Katu | Lagu-Ruksha | Ushana | Katu | - |
| **Bemithiriya** | Thiktha | Lagu- snigdha | Ushna | Katu | - |
| **Pawatta** | Katu-Thiktha-Kashya | Lagu-ruksha | Sheetha | Katu | Kapa shamaka |
| **Ithana** | Madura-Thiktha-kashaya | Lagu-snigda | Sheetha | Madhura | - |
| **Bulu** | Kashaya | Lagu-Ruksha | Ushna | Madura | Tridosha Shamaka |
| **Nelli** | Madura-Amla-katu-Thiktha-Kashaya | Lagu-Ruksha | Sheetha | Madura | Tridosha Shamaka |
| **Erabadu** | Thiktha-Katu | Lagu | Ushna | Katu | Kapha Vatha Shamka |
| **Karada** | Katu-Thiktha-Kashaya | Lagu-Thikshna | Ushna | Katu | Kappa Vatha Shamaka |
| **Siwiya** | Katu | Lagu-Ruksha | Ushna | Katu | - |
| **Muda Mahana** | Madura-Thiktha | Lagu | Ushna | Katu | - |
| **Enasal** | Madura-Katu | Lagu-Ruksha | Sheetha | Madura | |
| **Nerenchi** | Madura | Guru-Snigda | Sheetha | Madura | Vatha Piththa Shamaka |
| Divalabu | Katu | Lagu-snigda-Thikshna | Ushna | Katu | Vatha Piththa Shamaka |
| Puwak | Madura-Kashaya | Guru-Ruksha | Katu | Sheetha | Vatha Piththa Shamaka |
| Daluk | Katu | Lagu-Snigda-Thikshna | Ushna | Katu | Kapa Vatha hara |
| Bewila mul | Madura | Guru-Snigda-Pichchila | Shitha | Madura | Vatagna |

Table1: Pharmacognacy of Ingredients of paththu

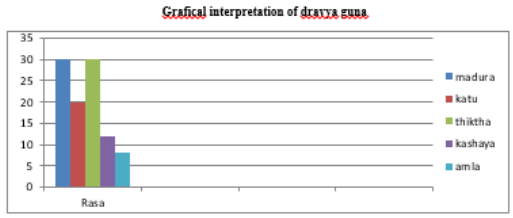


Table2: Graphical interpretation of Vipaka


Table3: Grafical Interpritation of vipaka


Table4: Grafical Interpritation of veerya



## *DISCUSSION*

Regarding the Property of Veerya ,It contains 78% of Ushna Veerya and 22% of Sheetha Veerya. Under the property of Vipaka 80% of drugs in Katu Vipaka , 20% madhura vipaka and 4% in Amla Vipaka.so the Ampla Vipaka herbs may used in very little.

There fore in amavatha shotha condition due to Madhura Rasa Vipaka-Ushna Veerya Vatha dosha theand Due to the thiktha Rasa ,ushana veerya kapha dosha became to shaman state.It clearly shows in the traditional ama vatha shotha paththu reduse the shotha condition by contributing shamana of Vata and kappa dosha very well.As the study of this case with the use of guli kalka sweda and the external paththu Incerase and normalise the decreased Agni and facilitated to digest the ama.there after shotha condition becomes

## *SUGESSIONS*

As the study of this case with the use of guli kalka sweda and the external paththu Incerase and normalise the decreased Agni and facilitated to digest the ama.there after shotha condition becomes normal there fore these traditional paththu and herbs can be used for the Ama vatha conditions very well and they can be cured without any side effects.Now a days western treatments were not given full recovery for the ama vatha conditions and with the above line of treatments it an be manage well therefore it is essential to use these kind of treatments for the ama vatha shotha conditions.

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# COMPARISON BETWEEN TWO DNA EXTRACTION METHODS FOR THE DETECTION OF LACTOBACILLUS IN YOGHURT

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## *ABSTRACT*

Probiotics are formulated into dairy products like yoghurt to enhance its functional properties. By definition probiotics are a group of live microorganisms, capable in providing health benefits to the host when delivered in adequate quantity. The genera most commonly used in this dietary preparation is Lactobacillus. The aim of this study was to evaluate the efficiency of the Lactobacillus DNA extraction from yoghurt using a commercial kit (PromegaTM WizardTM Genomic DNA purification kit) and an optimized boiled cell extraction method. A total of 5 isolates, obtained from 5 different commercial brands were characterized as Lactobacillus by considering physiological, morphological and biochemical characteristics. The DNA was then extracted by each of the two methods, and tested in genus-specific PCRs to confirm the presence of Lactobacillus. The DNA quantity and quality was determined by spectrometry. The most efficient method of extraction was the kit based method, in which a substantial DNA yield with purity was generated. The DNA yields from both extractions were further subjected to one-way analysis of variance (ANOVA) using SPSS (Version 21, IBM) statistical software, in which a significant difference [P (0.019)  0.05] was observed. In conclusion, the study emphasize on the use of appropriate DNA extraction protocol as the DNA purity and the quantity contributes to quality biological analyses such as polymerase chain reaction (PCR).

Key words: Yoghurt, Lactobacillus, DNA yield

## *INTRODUCTION*

Functional foods are considered a dietary requirement due to supplementary health benefits to an individual's health, in addition to its provision of incorporated nutrients. Among many other additives used in functional food production, probiotics are the most frequently used, precisely in fermented dairy products (Granto et al, 2010). Probiotics by definition are a group of live microorganisms, capable in providing health benefits to the host when delivered in adequate quantity. The term "probiotic'' was Greek originated and said to be invented by Ferdinand Vergin in 1954, which was further addressed in articles by Lilly and Stillwell (1965), Fuller (1989), Guarner and Schaafsma (1998) (Sanders, Merenstein, Merrifield and Hutkins, 2018).

The lactic acid bacteria (LAB) is one of the large group of microorganisms commonly preferred as potential pathogens in food industry. LABs ferment carbohydrate either homofermentatively or heterofermentatively to produce lactic acid as the major end product in which by-products contribute to the enhancement of

organoleptic properties of the food. LAB comprises of 16 genera, in which 12 are industrially exploited as starter cultures, including Lactobacillus, Streptococcus, Bifidobacterium, Carnobacterium, Aerococcus, Leuconostoc and Enterococcus (Zielińska and Kołożyn-Krajewska, 2018).

The largest genus within the LABs are Lactobacillus, which belongs to phylum Firmicutes and family Lactobacillaceae. They are known as gram positive, rod shaped, catalase-negative, non-spore forming anaerobic bacteria. Depending on the carbohydrate metabolism pathway, the bacterium is classified as obligate homofermentive, facultative heterofermentive or obligate heterofermentive. Lactobacillus share a diverse gram stain morphology like short plump rods, long slender rods, chains or palisades due to its species variations. Furthermore colony morphology varies on the cultured medium, like gray colonies on blood agar and white, mucoid colonies on MRS (Man, Rogosa, and Sharpe) (Karami et al., 2017; Goldstein, Tyrrell and Citron, 2015).In addition to its presence in commercial products, some acid-tolerant Lactobacillus are naturally found in the human gut. Therefore, its products are stabilized by the bile and acidic PH. Probiotic actions of Lactobacillus include strengthening of epithelial barrier, production of antimicrobials, immune system modulation, the inhibition of pathogen adhesion by strong adherence to the intestinal mucosa and concomitant elimination of the pathogens (Bull, Plummer, Marchesi and Mahenthiralingam, 2013). Intestinal barrier integrity is said to be stabilized by the modulation of the genes encoding tight junction proteins like cadherin and catenin, further more cytokine induced apoptosis is inhibited by activating anti-apoptotic proteins thereby, preventing intestinal disorders like inflammatory bowel disease. Presence of mucus adhesion-promoting proteins mediate the adhesion of the bacteria with the mucus. Furthermore, the stimulation of the release of mucin and defensins from the epithelial cells inhibit the adherence of gastrointestinal pathogens. The productions of antimicrobials also known as bacteriocins, bacteriocins induce pore formation in the pathogen's cell membrane, causing destabilization of the membrane, resulting cell lysis or involve in the inhibition of the pathogenic cell wall synthesis (Bermudez-Brito et al., 2012). In the regard of the immunomodulation, down regulation of toll-like receptors, inhibition of NF-kB signaling in the innate immune system and up regulation of NLR expression involve in apoptosis, contributes to the regulation and suppression of intestinal inflammation (Wells,2011).

Probiotics delivery by non-conventional food based products like yoghurt, cheese and chocolate as a method of supplementation of intestinal favorable bacteria is preferred over conventional pharmaceutical formulations due to easy availability, convenience and the providence of accurate physiological conditions for the bacterial survival (Govender et al., 2013). Among commercial products, yoghurt have been commonly used for the probiotic delivery. The yoghurt contain a mixture of milk, water, protein, fats, carbohydrates and bacterial cultures. The synergistic relationship between the components of the dairy product and probiotic cultures marks fermented dairy products as a good candidate. The ability of the yoghurt to deliver probiotics is demonstrated by Hemsworth, Hekmat and Reid, 2011; where the incoporated Lactobacillus strain count elevated upon administration of the yoghurt. Furthermore, Marafon et al., in 2011 demonstrated the methods of optimization of the yoghurt as a delivery system. Probiotic yoghurt usually contain a combination of Lactobacillus and

streptococcus or Bifidobacterium and Lactobacillus as starter cultures (Routray and Mishra, 2011). A study evaluated the combined effects of Bifidobacterium and Lactobacillus in yoghurt which produced satisfactory results in high caries individuals upon short-term consumption (Bafna et al., 2018). Similarly the efficacy of the two probiotics in lowering serum cholesterol level was studied in which demonstrated positive results, providing evidence of the Lactobacillus in the health management (Rerksuppaphol and Rerksuppaphol, 2015). This study evaluate the efficiency of the two DNA extraction methods for Lactobacillus and emphasize on the necessity of high purity and yield of DNA for successful PCR.

## *METHODOLOGY*

Sample collection and preparation

Five yoghurt samples (A-E) from different brands were obtained. Approximately 10g of each sample was aseptically transferred into the beaker. The samples was then gently stirred to concoct a homogenous mixture. The above step was carried out for all the samples.

**Culturing of the bacteria on MRS agar**

A loop full of sample was aseptically obtained and streaked on agar (refer appendix) using quadrat technique. The petri plates was then incubated at 37□C for 24-48 hours before observing the morphological features.

**Gram staining**

A loop full of distilled water was placed on the glass side and an isolated bacterial colony was introduced aseptically into the water drop. The bacteria was evenly dispersed to form a smear. The smear was then air dried and heat fixed before proceeding with the staining. The smear was initially stained with crystal violet for 1 minute, followed by Gram's iodine for 1 minute, decolorizing agent for 3seconds and safranin for 1 minute. After each step of flooding the slide with the reagents and its incubation, the slide was thoroughly washed in gentle stream of water and blotted and air dried before observing under 100X magnification.

**Catalase test (Slide test)**

Small amount of bacterial colony was aseptically transferred to a glass slide using a loop. A drop of 3% H2 O2 was introduced to the bacteria on the slide, and then observed for fizzing (formation of bubbles)

**Sub culture**

Into the prepared broth (refer appendix), pure bacterial colony was introduced and incubated at 37□C for 24-48 hours. The aforementioned step was repeated for all the five samples. DNA extraction with Modified boil cell method by Perera and Weerasooriya, 2019.

5ml of bacterial broth was centrifuged at 4000rpm for 15minutes. Into the pellet 100µl of TE buffer was added and left in the water bath for 20 minutes, followed by quick freezing at -20□C for 20 minutes. The sample was then centrifuged at 4000rpm for 10 minutes. Supernatant was transferred into a new tube and 60µl of 10mg/ml lysozyme was added along with 5µl of 10mg/ml proteinase K and 20µl of 10% SDS. After the sample incubation at 37□C for 20 minutes, 0.5ml of saturated NaCl solution was added and mixed vigorously followed by a centrifugation at 13000rpm for 3 minutes. The supernatant was carefully obtained and transferred into a new microcentrifuge tube. 100µl of cold 100% ethanol was added and quick spinned. Supernatant was discarded, into the pellet 200µl of 70% ethanol was added to wash the pellet. The aforementioned step was repeated twice. The tubes was then allowed to air dried thoroughly in order to evaporate all the ethanol. DNA pellet was dissolved in 100µl of TE buffer and stored at -20ºC in the refrigerator.

DNA extraction with PromegaTM WizardTM Genomic DNA purification Kit

1ml bacterial broth was centrifuged at 13000rpm for 2 minutes and supernatant was discarded. 480µl of 50mM EDTA was then added along with 120µl of lytic enzyme and mixed gently using the pipette, followed by an incubation at 37□C for 45 minutes and centrifugation at 13000rpm for 2 minutes. The supernatant was then discarded. The sample was re-suspended with 600µl of nuclei lysis solution and incubation was carried out at 80□C for 5 minutes. After allowing to cool down to room temperature, 3µl RNase solution was added and mixed by inverting the tube several times. The sample was then incubated at 37□C for 60 minutes, after cooling down to room temperature, 200µl of protein precipitation solution was added and vigorously vortexed for 20 seconds. The sample was incubated on ice for 5 minutes followed by a centrifugation at 13000rpm for 3 minutes. Supernatant was carefully obtained and transferred into a new tube. 600µl of isopropanol was added and mixed by inverting the tube until thread-like DNA strands were visible. The sample was then centrifuged at 13000rpm for 2 minutes and the supernatant was discarded. 600µl of 70% ethanol was added and centrifuged at 13000rpm for 2 minutes. The ethanol was left to aspirate and air dried overnight. 100µl of rehydrating solution was added and incubated at 65□C for 1 hour. The solution was periodically mixed and stored at -20□C.

**Quantification of the extracted DNA**

10µl of both boil cell and kit based extracted DNA was diluted in 2990µl of TE buffer. The absorbance was measured at 260nm, 230nm and 280nm in triplicates (Refer appendix).

DNA concentration, Yield and purity was calculated using below equations (Refer appendix);

DNA concentration (µg/µl) = absorbance at 260nm x dilution factor x 50µg/µl

DNA yield (µg) = DNA concentration (µg/µl) x DNA stock volume (100µl)

DNA purity = A260/A280 and A260/A230 A=absorbance

Genus specific identification of Lactobacillus using PCR

Boil cell and kit based extracted DNA was amplified using genus specific primers (Table 1) and the PCR mix was prepared accordingly (Table 2).

Table 2. PCR genus-specific primers sequences

| Primer | Sequence (5'-3') | Expected size of the amplicon | Reference |
|---|---|---|---|
| LactoF | TGGAAACAGRTGCTAATACCG | 233bp | Byun *et al.*, 2004 |
| LactoR | GTCCATTGTGGAAGATTCCC | | |

*Table 2. Reagents and volumes required for the preparation of the PCR mixture for Lactobacillus identification*

| **Reagents** | **Volume for 1 reaction (µl)** | **Final Concentration** | **Volume for 8 reactions (µl)** |
|---|---|---|---|
| 5X PCR buffer | 5µl | 1X | 40µl |
| 25Mm $MgCl_2$ | 1.5µl | 1.5mM | 12µl |
| 10mM dNTPs | 0.5µl | 0.2mM | 4µl |
| 2µM Lacto F primer | 2.5µl | 0.2µM | 20µl |
| 2µM Lacto R primer | 2.5µl | 0.2µM | 20µl |
| 5U/µl Taq DNA polymerase enzyme | 0.25µl | 0.05U/µl | 2µl |
| Sterilized water | 11.75µl | | 94µl |
| DNA | 1µl | 100 ng/µl | |
| **Total volume** | 25µl | | 200µl |

The reaction mixture of 25µl was prepared for 8 reactions, 5 samples (A-E), positive and negative controls. The PCR was carried out according to the cyclic conditions given in table 3.

*Table 3. Cyclic conditions for the PCR*

| **Process** | **Temperature** | **Time** | |
|---|---|---|---|
| Initial denaturation | 94◦C | 5 minutes | |
| Denaturation | 94◦C | 1 minute | 35 cycles |
| Annealing | 62◦C | 1 minute | |
| Extension | 72◦C | 2 minutes | |
| Final extension | 72◦C | 12 minutes | |
| Final hold | 4◦C | ∞ | |

Visualization of PCR products

Into 2% agarose gel, 2µl of 50bp DNA ladder was loaded into the first slot separately followed by addition of 7µl of each PCR products (A-E, and N) consecutively. Positive control was loaded adjoining to the negative control (N). The gel was initially allowed to run at 45V for 35 minutes and then switched to 50V for 25minutes. Finally the gel was observed under the UV light.

## *DATA ANALYSIS*

DNA yield obtained from modified boiled cell and kit based methods was compared using one way ANOVA, and the P value was calculated at 0.05 significance level.

## *RESULTS*

Culturing of the bacteria on MRS agar

The bacterial culture was incubated at 37□C for 24-48 hours and the colony morphology was observed as below (Figure 1)

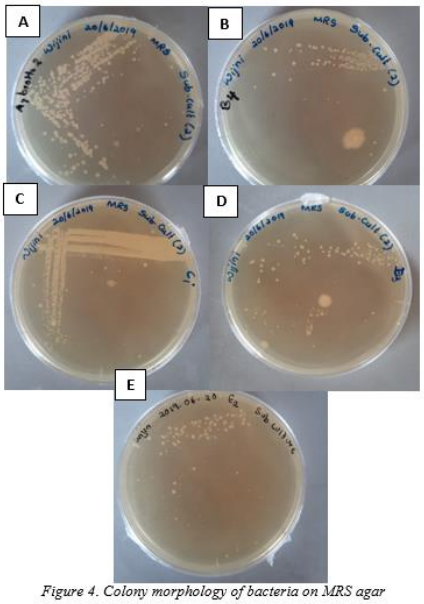



*Figure 4. Colony morphology of bacteria on MRS agar*

Colonies with creamy grey colour, round in form, entire in margin and raised in elevation was noted on the surface of the medium in all the five samples. Contamination was observed in samples B and D

**Gram staining**

The morphological features of the isolated bacteria were further examined by Gram staining as shown below (Figure 2)

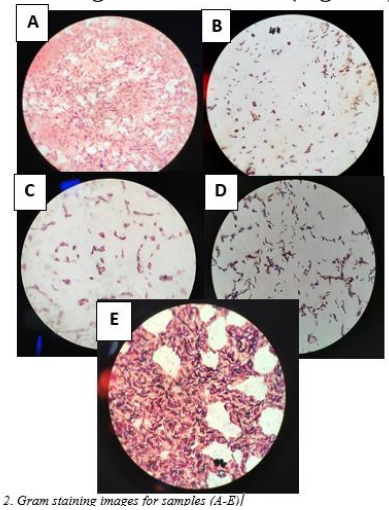



*Figure 2. Gram staining images for samples (A-E)*

Rod shaped, purple coloration was observed in all the five samples indicating the presence of Gram-positive bacillus bacteria

**Catalase Test**

The presence of catalase enzyme in the bacterial isolate was determined by the catalase test (Figure 3)

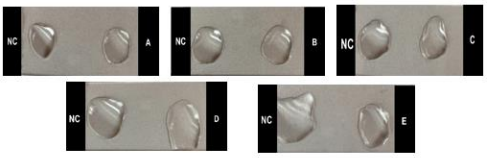
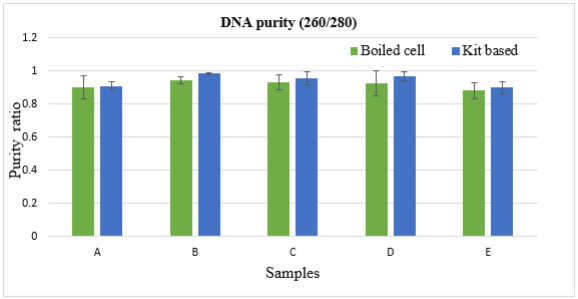



*Figure 3. Slide method for catalase test*

The mixture in all the five samples produced no effervescence, thus, identifying the bacteria as catalase-negative.

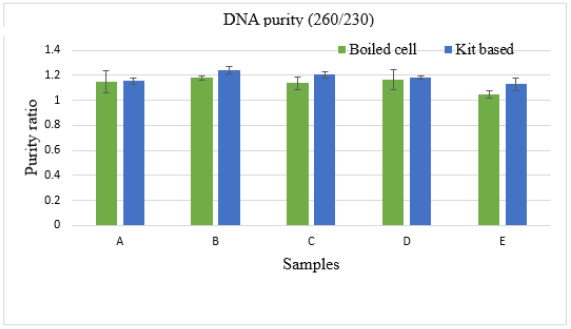
DNA purity (260/280)




Graph 1. DNA purity (260/280) obtained from the two extraction methods

The mean values of the ratio A260/A280 was high in kit based method. The sample B depicts the highest purity and Sample E with lowest purity in both the extraction methods

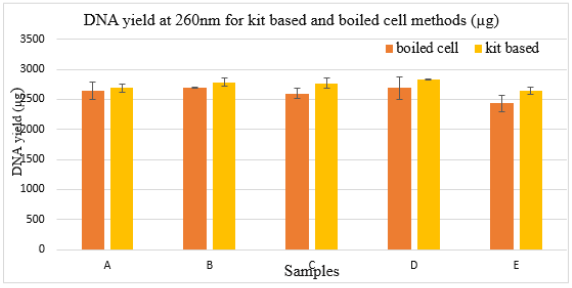
DNA purity (260/230 Ratio)




*Graph 2. DNA purity (260/230) obtained from the two extraction methods*

The mean values of the ratio A260/A230 was high in kit based method. The sample B depicts the highest purity and sample E with lowest purity in both the extraction methods

The DNA yield




*Graph 3. DNA yield obtained from the two extraction methods.*

A higher DNA was obtained from kit based than the modified boiled cell method. Sample D depicts the highest yield among all the samples in kit based whereas, sample B for modified boiled cell method.

## *DATA ANALYSIS*

DNA yield from the two extraction methods was compared using one way ANOVA generated by SPSS (Table 4)

**ANOVA : DNA yield**

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 96787.200 | 1 | 96787.200 | 6.185 | .019 |
| Within Groups | 438185.100 | 28 | 15649.468 | | |
| Total | 534972.300 | 29 | | | |

*Table 4. Statistical analysis for the DNA yield with the extraction method*

P value (0.019) is less than 0.05.Therefore, there is a significant difference between DNA yields obtained from the two extraction methods.

Genus specific identification using PCR

The DNA from both extraction methods was amplified using genus specific primers and the gel images was obtained as below (Figure 4 and Figure 5).

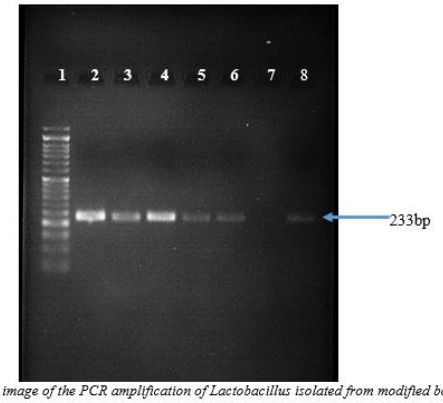



*Figure 4. Gel image of the PCR amplification of Lactobacillus isolated from modified boiled cell method (L1- 50bp DNA ladder, L 2-L6-Samples, L7-Negative control, L8-Positive control)*

All the produced amplicons of 233bp band size which was similar to the positive control and the negative control was free from any bands. Furthermore variations in band intensities was noted.

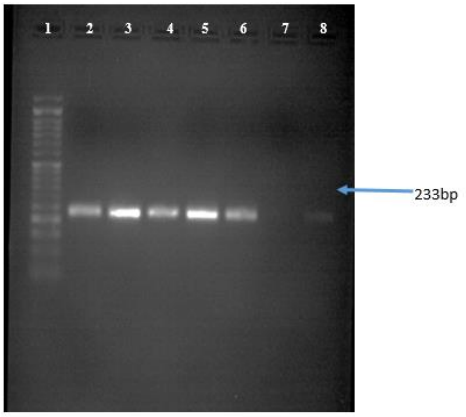



*Figure 5. Gel image of the PCR amplification of Lactobacillus isolated from kit based method (L1- 50bp DNA ladder, L 2-L6-Samples, L7-Negative control, L8-Positive control)*

All the produced amplicons of 233bp band size which was similar to the positive control and the negative control was free from any bands. Furthermore variations in band intensities was noted.

## *DISCUSSION*

Probiotics are a group of live microorganisms, defined by their genus, species and strain designations. Upon administration of adequate quantity, they are capable in providing health benefits to the host by restoring the gut floral balance (Sanders, Merenstein, Merrifield and Hutkins, 2018). The aim of this study was to isolate Lactobacillus from yoghurt by two extraction methods and evaluate the DNA generated with regards to yield and purity

The isolates of each yoghurt sample was phenotypically identified. All samples displayed creamy-grey mucoid colonies on MRS medium and exhibited a colony morphology of round in form, entire in margin, raised in elevation as observed in Lactobacillus. Even though MRS is a selective culture medium designed to encourage Lactobacillus growth, Streptococci growth was noted with colonies of creamy white in colour, round in form, entire in margin and convex in elevation. The streptococcus growth could to be inhibited by lowering the PH level of the medium. Similar results was obtained by Guevarra and Barraquio, 2016. Fungal growth was noted in samples B and D which may be due to the sample originated contamination. This could be prevented by the addition of antifungal compounds like cycloheximide (Karami et al., 2017) (Figure 1). Considering the gram staining (Figure 2), the bacteria in all five samples appeared bacillus in shape with bluish-purple stain. The gram positive rod shaped bacterial isolates were determined as representative of genus Lactobacillus (Qian et al., 2018). Furthermore, the catalase test (Figure 3) was negative as no bubbles was evolved, thereby confirming the presence of Lactobacillus with the aid of the results obtained by Islam et al., 2016.

Isolation of DNA with high yield and maximum purity are the two main features of a good extraction method. The purity was assessed by spectroscopy using both A260/A280 and A260/230 absorbance ratios (Graph 1 and 2). As a guideline, a good quality DNA should possess a value from 1.8-2.0 for A260/A280, the values above or below the reference range indicative of contamination with RNA and protein respectively. It has been reported that DNA absorption depends on the pH of solution, acidic pH (low ratio) and basic pH (high ratio). Therefore, lactic acid produced by Lactobacillus is suspected to cause lower A260/A280 ratio (Lucena-Aguilar et al., 2016). Whereas, in A260/A230 any value below the range 1.8-2.2 indicates contamination with phenol, salt, proteins or polysaccharides

(Olson and Morrow, 2012). Even though both extraction methods failed to achieve the criteria, upon value comparison, less contamination was noted in kit based extraction. This may be due to the additional step of protein precipitation after the RNase treatment both removing contaminants, especially proteins (Abed, 2013). Even though, the extracted DNA from both methods lacked purity, the DNA was used for the PCR to detect bacteria, in which reliable bands were obtained indicating the presence of intact DNA (Abdulamir, Yoke, Nordin and Baker, 2010).

The quality and yield of DNA is said to have a great influence on PCR process. The result in Graph 3, demonstrated the DNA extracted from kit protocol was higher than that of boiled cell. Moreover, the statistical analysis (Table 4) indicate a significant difference between the yield obtained by both extraction methods, as the p value $<0.05$ confirming the observation in the graph. According to Becker et al., 2016 Wizard genomic purification kit produced the highest yield among five commercially available kits for bacterial chromosome and plasmid DNA extractions. An adequate yield could be possible due to the addition of lysozyme in boiled cell method. Lysozyme is a best known muramidase, which efficiently hydrolyses 1, 4-beta glycosidic linkages present in the peptidoglycan layer of the gram-positive bacterial cell wall (Bag et al., 2016). A previous study conducted to determine the yield and quality of bacterial DNA extract from human oral rinse samples demonstrated a direct relationship between cell lysis method and the DNA yield. Here, zirconium beads cell lysis together with lysozyme was emphasized as an effective method for the bacterial DNA extraction (Sohrabi et al., 2016). However, in contrast, another similar study notify the application of enzymatic digestion (Lysozyme, mutanolysin and lysostaphin) alone than using bead-beading for greater DNA yield (Rosenbaum et al., 2019). The kit protocol of this study utilize both lysozyme and EDTA. According to Moore et al., 2004, lysozyme in combination with EDTA considered efficient in disrupting the bacterial cell wall. The modified boiled cell method produced better yield than the native boiled cell (Perera and Weerasooriya, 2019). Even though, the modified boiled cell method is a rapid, cost-effective, simple method for DNA isolation, due to the rigid nature of gram-positive bacterial cell wall, the yield generated was low (Ahmed, Asghar and Elhassan, 2014). However, the DNA extraction by boiling was sufficient for the PCR amplification of Lactobacillus DNA. Another study conducted to optimize and evaluate three Lactobacillus extraction methods confirm a higher yield from kit than boiled cell and phenol-chloroform methods, here an additional proteinase K step was included in the kit protocol which aided in further lysis of the cell (Abdulla, 2014).

A PCR was carried out to further confirm the results obtained from biochemical tests. The positive control containing lactobacillus produced an amplicon of 233bp (Byun et al., 2004). Similar bands at 233bp was observed for all the five samples each extracted from both the extraction methods. According to these results, all the samples were positive for Lactobacillus. Moreover, the negative control showed no band, indicating free from contamination. In this study, the PCR result of 233bp band, was in accordance with a study conducted by Senthilkumar et al., 2018).

## *CONCLUSION*

In this study the microbiological and molecular techniques were used to determine the presence of Lactobacillus in a mixed microbial population. Among the

two extraction methods used, the PromegaTM WizardTM Genomic DNA purification kit was efficient over modified boiled cell by producing considerable DNA yield and purity. The variations in the DNA yield and the purity with regard to the boiled cell could be improved by further optimizing the aforementioned protocol.

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# IDENTIFICATION OF LACTOBACILLUS IN FERMENTED CHEESE AND THE ANALYSIS OF THEIR ANTIBIOTIC RESISTANCE

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## *ABSTRACT*

Probiotics are living microorganisms, that can improve health by providing benefits beyond that of the traditional nutritional value when consumed in required amounts. They are present in various fermented foods, including cheese where Lactobacillus is one of the most commonly found species. This study was designed to isolate and evaluate the antibiotic resistance patterns of Lactobacillus present in ten commercially available Cheddar (n=5) and Gouda cheese (n=5) samples. The Lactobacillus from homogenized cheese samples were isolated on Lactobacillus specific MRS agar, and phenotypic identification was achieved by morphological and biochemical investigations such as colony morphology, gram's staining and catalase test. The genomic DNA from isolated Lactobacillus colonies were extracted from the Wizard genomic DNA purification kit and modified boiled cell method. Extracted DNA was quantified using the spectrophotometer. Results revealed that the DNA yields significantly varied between the two methods ($P < 0.05$). PCR based genotypic detection of Lactobacilli using genus-specific primers revealed the presence of bands in all samples. Furthermore, in a PCR assay for the detection of antibiotic resistance genes, the erm(B) gene was found in 1 sample (n=1) and no sample was positive for the tet(M) gene (n=0). In conclusion, the wizard method was confirmed as the most efficient extraction method since it indicated higher DNA yields and purity. Screening of antibiotic resistance of probiotics used in the food industry is essential for the safety of probiotic food products as resistance genes can be transferred to pathogens.

Keywords: Probiotics, Lactobacillus, Cheese, Antibiotic Resistance

## *INTRODUCTION*

Probiotics are viable microorganisms which when administered in appropriate amounts exert beneficial effects on the consumer by improving the gastrointestinal flora in the GIT (Kechagia et al., 2013). These bacteria are non-toxic and are categorized as 'GRAS' organisms and can be safely used in the processing of food and clinical applications. They are highly used for fermenting food or added as dietary supplements to food, especially for dairy products as they are able to effectively carry these probiotic microorganisms especially species of Lactobacillus and Bifidobacterium. Common food types that contain probiotic bacteria include fresh milk, and fermented food such as olives, pickles yoghurt, curd and kefir (Rezac et al., 2018). Some of these organisms reside in the human gut, naturally associated with the

gastrointestinal microbiota. These beneficial probiotic microorganisms promote digestion, strengthen the immune system, inhibit the growth of pathogenic bacteria, alter intestinal flora equilibrium and increase resistance to infection. In addition, probiotics is therapeutically proven to reduce blood cholesterol, prevent cancer, improve lactose tolerance, prevent or reduce the effects of Crohn's disease, acute diarrhoea, and constipation as well as candidiasis urinary tract infection (Reid, 1999). A study by Ghoneum and Gimzewski, (2014), found that Lactobacillus species in kefir possessed an apoptotic effect on cell lines of myeloid leukaemia. Yang et al., (2014), showed that the clinical improvement of children with atopic dermatitis is associated with probiotic supplements containing Lactobacillus species.

The genus Lactobacillus is gram-positive, catalase-negative, non-spore producing bacteria which are appeared as non-motile thin rods in different lengths (Rafieian-Kopaei et al., 2017). It is also known as a member of lactic acid bacteria as they produce lactic acid as a sole or primary product of carbohydrate metabolism. In addition, Lactobacillus is fermentative, facultative anaerobes or aerotolerant, aciduric or acidophilic bacteria with below 50% mol (33% - 50%) G + C content (Tannock, 2004; Slover and Danziger, 2008). Currently, more than 80 species of Lactobacilli have been identified and, L. casei, L. acidophilus, L. fermentum, and L. salivarius are the commonest Lactobacilli found in human GI tract (Walter, 2008). Probiotics confer beneficial effects on human health via several mechanisms. They contribute to the enhancement of the intestinal epithelial barrier, the only physical barrier against invasion of the tissue's underneath by potential pathogens. For instance, Lactobacilli regulate the genes encoding the proteins responsible for cell adhesion. Probiotics adhered to the intestinal mucosa causes alterations in intestinal mucins, to facilitate pathogen binding and also trigger the epithelial cells to secrete defensins, to fight against pathogenic bacteria. Moreover, the production of lactic and acetic acid from probiotic bacteria can result in environmental modification to make it less suitable to the competitive pathogens thereby, excluding pathogenic microbes (Bermudez-Brito et al., 2012). The major role is played by antibacterial substances including bacteriocins produced by the probiotic bacteria, which are generally considered alternative to antibiotics that act against the growth of several pathogenic bacterial strains (Eid et al., 2016). Thereby, can improve host health by fighting off pathogenic organisms and treating bacterial intestinal infections. Jin et al. (1996) reported that Lactobacillus species from chicken intestine inhibited strains of E. coli and Salmonella. Similarly, antimicrobial activity of bacteriocin produced by probiotics showed that, Lactobacillus species had strong antibacterial effects against pathogenic bacteria (Tambekar and Bhutada, 2010).

Cheese is the most widely consumed milk product worldwide. Various Lactobacilli are used in cheese making. They aid in the development of the cheese flavor when used as adjunct cultures (Stefanovic et al., 2018). Lactobacilli rely on other potential sources of energy such as metabolizing peptides, amino acids, sugars released from enzymatic hydrolysis of casein, and products of degrading starter bacteria in order to sustain growth in cheese depleted of fermentable carbohydrates (Peterson and Marshall, 1990). The high-fat content and buffering capacity of the cheese provides additional advantages for the survival of probiotics in the gastric environment (Gomes da Cruz et al., 2009).

Lactobacilli resistance to antibiotics is achieved through acquired and intrinsic mechanisms. Intrinsic resistance is the

innate ability of the bacteria to tolerate the antibiotic activity through its inherent structural or functional characteristics. Vancomycin resistance of Lactobacilli is the best example. It inhibits peptidoglycan, in the bacterial cell wall, thus, inhibiting growth (Anisimova and Yarullina, 2018). Studies on L. acidophilus and L. reuteri species demonstrated that all L. reuteri species were resistant to vancomycin whereas, only a few strains of L. acidophilus were vancomycin resistant. Acquired resistance of bacteria is either due to the occurring of spontaneous gene mutations or horizontal gene transfer, which exerts a threat on the non-pathogenic strains. In the case of intrinsic antibiotic resistance, antibiotic resistance genes in Lactobacilli are not transferred to the intestinal microflora unlike the acquired resistance, which can result in the exchange of resistance genes from Lactobacilli to the intestinal flora and virulence genes vice versa. This can completely modify the genotypic profile of the microbes (Jose et al., 2015). Furthermore, in Lactobacillus species, genes conferring resistance to tetracycline and erythromycin through various mechanisms have been detected, of which tet(M) and erm(B) genes are the two commonly found genes (Gevers et al., 2003). These genes are considered potentially transferable to plasmids and integrons in pathogenic microbes within the host. The emergence of such bacteria can pose a serious global threat (Peterson and Kaur, 2018).

Lactobacilli have been observed to be resistant to a wide range of antibiotics. Thereby, has a high possibility of transferring the resistance to the consumers' intestinal flora and be horizontally transferred to a pathogen. The current study was aimed to characterize the most efficient method of DNA extraction and assess the antibiotic resistance, so as to evaluate the safety of the cheese samples to be consumed.

## *METHODOLOGY*

**Sample Collection and Preparation**

Five cheddar cheese samples (A-E) and five gouda cheese samples (F-J) of different brands were purchased from Colombo and under aseptic conditions, 10g of each cheese samples were homogenized in 10ml of autoclaved distilled water in beakers.

Isolation of Lactobacillus on MRS Agar

20 ml of prepared MRS agar was poured into the petri dishes and allowed to solidify. Then, under aseptic conditions loop full of samples were inoculated on solidified MRS agar plates according to the quadrant streaking method. The petri plates were incubated at 370C for 48 hours.

**Gram Staining**

After 48 hours, colony morphology of the bacterial growths was observed, and bacterial smears were prepared on glass slides. The heat fixed smear was flooded with crystal violet for 1 minute, Gram's iodine for a minute, decolorizer for 3 seconds and safranin for 45 seconds. After each step of flooding, the slide was washed with distilled water.

**Catalase Test**

Bacterial smears were prepared using marked colonies on the glass slides and added a drop of 3% H2O2 on the smears.

**Sub-culturing of pure colonies**

The gram-positive and catalase negative colonies were sub-cultured in 10 mL Lactobacillus MRS broth, incubated at 37°C for 2 days and stored at 4 °C.

**DNA extraction via Modified Boiled cell method**

5ml of broth from each sample were taken into flacon tubes (15ml) and centrifuged at 4000rpm (15 minutes). Then, the supernatants were discarded and added 100µl of TE buffer. Thereafter, the falcon tubes were kept in the water bath at 1000C (20 minutes) and quickly froze at -200C (20 minutes). After that, the frozen

samples were thawed and centrifuged at 4000rpm (10 minutes). Then the supernatants were transferred to another Eppendorf tubes (1.5ml) and added 60µl of lysozyme (10mg/ml), 5µl of proteinase K (10mg/ml) and 20µl of 10% SDS. After that, incubated at 370C (1hour) and added 0.5ml of NaCl. Then, the Eppendorf tubes were shaken vigorously and centrifuged at 13000rpm (3 minutes). Thereafter, transferred the supernatants into new Eppendorf tubes and added 100µl of cold 100% ethanol. Then the samples were mixed and spun for few seconds, and supernatants were removed. After that, added 200µl of 70% ethanol and spun and removed the supernatants (repeated 2-3 times). After that DNA pellets were airdried and added 100µl of TE buffer. Finally extracted DNA samples were stored at -200C.

DNA extraction via Wizard® Genomic DNA Purification Kit

1 mL broth of sample A was added into an eppendorf, centrifuged at 13,000 rpm for 2 minutes and the supernatant was discarded. To it, 480 µl EDTA and 120 µl lysozyme was added, incubated for 60 minutes at 37°C, centrifuged at 13,000 rpm for 2 minutes and the supernatant was discarded. 600 µl nuclei lysis solution was added and incubated at 80°C for 5 minutes. After cooling at RT, 3 µl RNase was added, inverted a few times and incubated at 37°C for 1 h. To it, protein precipitation solution of 200 µl was added, vortexed for 20 seconds, incubated on ice for 5 minutes and centrifuged at 13,000 rpm for 3 minutes. The supernatant was transferred into an eppendorf containing 600 µl isopropanol, gently mixed, centrifuged at 13,000 rpm for 2 minutes and the supernatant was removed. The pellet was washed with 600 µl of 70% ethanol. Ethanol was aspirated after centrifuging at 13,000 rpm for 2 minutes. The pellet was left to air dry and was re-hydrated by adding 100 µl DNA rehydration solution. The mixture was then incubated at 65°C for 1 hour and the DNA extracted was stored at 2 - 8°C. The above procedure was repeated for the rest of the samples.

**Quantification of Extracted DNA**

10µl of extracted DNA samples were diluted in 2990µl of TE buffer and measured the absorbance at 260nm, 230nm, and 280 nm in triplicates. Then, calculated the DNA purity, concentration, and DNA yield, using below equations for both extraction methods.

DNA Purity = A260/280 and A260/230

DNA concentration (µg/µl) = (A260 in OD units x 50 µg/ml x DF) /1000.

DNA yield (µg) = DNA concentration (µg/µl) x amount of DNA kept as stock

Then, DNA samples were diluted up to 100ng/µl by adding adequate amount TE buffer for the usage of PCR

**Identification of genus Lactobacillus by PCR**

The extracted DNA from both methods were amplified using genus-specific primers (Table 02). The PCR master mix was prepared according to the below table (Table 01)

*Table 01. Materials required for preparation of PCR master mix for five samples, negative control, and positive control.*

PCR

| Component | Stock Concentration | Working Concentration | Volume (µl) | |
|---|---|---|---|---|
| | | | Per one reaction | Per 16 reactions |
| PCR buffer | 5X | 1X | 5.0 | 80.0 |
| $MgCl_2$ | 25 mM | 1.5 mM | 1.5 | 24.0 |
| Forward primer | 2 µM | 0.2 µM | 2.5 | 40.0 |
| Reverse primer | 2 µM | 0.2 µM | 2.5 | 40.0 |
| 10 mM dNTP | 10 mM | 0.2 mM | 0.5 | 8.0 |
| *Taq* polymerase | 5 U/µl | 0.05 U/µl | 0.25 | 4.0 |
| DNA Template | - | 100 ng/µl | 1.0 | 16.0 |
| Autoclaved distilled water | - | - | 11.75 | 188.0 |
| Total volume | - | - | 25.0 | 400.0 |

Master mix was prepared for sixteen reactions (10 samples, 2 negative and 2 positive controls) and aliquoted 12.25µl of master mix into PCR tubes. Thereafter, DNA samples, Taq polymerase, and distilled water were added into the corresponding PCR tubes. 1µl of distilled water was added to the negative control instead of DNA. Then PCR was carried

out according to the cyclic conditions given in Table 03.

*Table 02. Lactobacillus genus specific primer sequences.*

| Primer | Primers (5' – 3') | Amplicon Size | Reference |
|---|---|---|---|
| Forward primer | 5' TGGAAACAGRTGCTAATACCG 3' | 233bp | Byun *et al.*, (2004) |
| Reverse primer | 5' GTCCATTGTGGAAGATTCCC 3' | | Byun *et al.*, (2004) |

*Table 03. Thermal cycle for Lactobacillus primers*

| Steps | Temperature (°C) | Time | Cycle No |
|---|---|---|---|
| Initial Denaturation | 94°C | 5minutes | |
| Denaturation | 94°C | 1 minute | 35 cycles |
| Annealing | 62°C | 1 minute | |
| Extension | 72°C | 1minute | |
| Final extension | 72°C | 12 minutes | |
| Infinite hold | 4°C | œ | |

Detection of antibiotic-resistant genes

Lactobacillus positive samples were amplified using tet(M) and erm(B) specific primers (Table 05).

The PCR master mix was prepared following the same procedure mentioned in Table 02 and PCR was performed using tet(M) and erm(B) primers (Table 04).

*Table 04. Primers for PCR detection of tet(M) and erm(B) antibiotic resistance genes.*

| Gene | Primers (5' – 3') | Amplicon Size | Reference |
|---|---|---|---|
| *erm(B)* | Forward: 5'CATTTAACGACGAAACTGGC 3' Reverse: 5' GGAACATCTGTGGTATGGCG 3' | 405 bp | Gad, Abdel-Hamid, and Farag, (2014). |
| *tet(M)* | Forward: 5'GGTGAACATCATAGACACGC 3' Reverse: 5'CTTGTTCGAGTTCCAATGC 3' | 401 bp | Gad, Abdel-Hamid, and Farag, (2014). |

*Table 05. Thermal cycle for erm(B) primers*

| Steps | Temperature (°C) | Time | Cycle No |
|---|---|---|---|
| Initial Denaturation | 94°C | 5minutes | |
| Denaturation | 94°C | 1 minute | 35 cycles |
| Annealing | 55°C | 1 minute | |
| Extension | 72°C | 1minute | |
| Final extension | 72°C | 12 minutes | |
| Infinite hold | 4°C | œ | |

*Table 06. Thermal cycle for tet(M) primers*

| Steps | Temperature (°C) | Time | Cycle No |
|---|---|---|---|
| Initial incubation | 94°C | 5minutes | |
| Denaturation | 94°C | 1 minute | |
| Annealing | 52°C | 1 minute | 35 cycles |
| Extension | 72°C | 1minute | |
| Final extension | 72°C | 12 minutes | |
| Infinite hold | 4°C | œ | |

**Agarose gel electrophoresis**

The PCR products were visualized using prepared 2% agarose gel electrophoresis. 50 bp ladder (2µl), PCR products (A-J) (7µl), negative and positive controls (7µl) were loaded into the wells according to the mentioned order and gel was run under 45V for 35 minutes and 50V for 25 minutes. Finally, bands were visualized under UV illuminator

## *DATA ANALYSIS*

The DNA yields obtained from both the extraction methods were compared using one-way ANOVA, and P-value was calculated using SPSS version 25.

## *RESULTS*

Isolation of Lactobacillus in MRS agar

The morphological features of the bacterial cultures incubated for 48 hours are shown in figure 01.

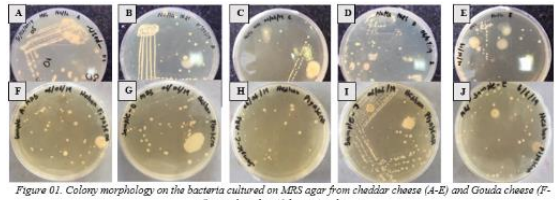



*Figure 01. Colony morphology on the bacteria cultured on MRS agar from cheddar cheese (A-E) and Gouda cheese (F-J) samples after 48 hours incubation.*

The shiny whitish cream color, mucoid, and rounded colonies with wavy margins were observed from all ten samples, and sample D showed a comparatively higher number of colonies. Some contaminations were observed from several samples.

Gram Staining

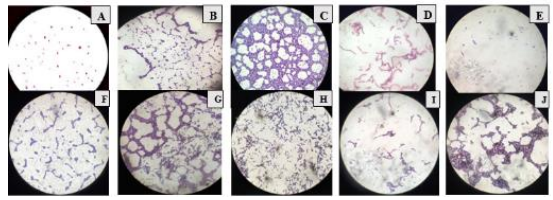


Figure 02. Gram Staining for possible Lactobacillus colonies from cultured cheddar cheese (A-E) and Gouda cheese (F-J) samples

The gram staining of the selected colonies from all the samples indicated the appearance of purple color, rod-shaped (bacilli) bacteria of varied lengths in chains and palisades, reveling the presence of gram-positive bacteria.

**Catalase Test**

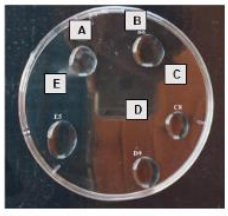



*Figure 03. Results of the catalase for selected colonies from cultured cheddar cheese sample*

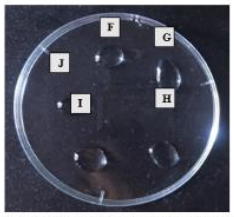
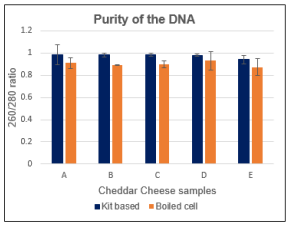


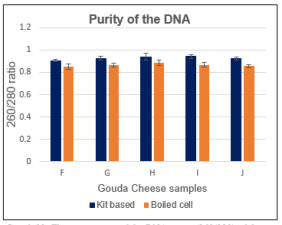

*Figure 04. Results of the catalase for selected colonies from cultured gouda cheese sample*

No air bubble formation observed in any sample indicating the presence of catalase negative bacteria in the selected colonies from each sample.

**Purity of the DNA (260/280 Ratio)**



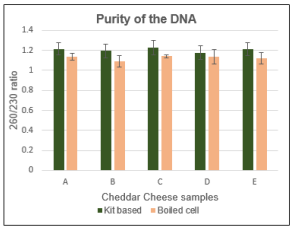

*Graph 01. The comparison of the DNA purity (260/280) of the extracted DNA from cheddar cheese samples via kit-based method and modified boiled cell method.*



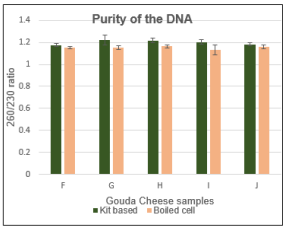

*Graph 02. The comparison of the DNA purity (260/280) of the extracted DNA from Gouda cheese samples via kit-based method and modified boiled cell method.*

The DNA samples extracted from both methods indicated lower purity. However, the DNA purity of the samples, extracted from the kit-based method were higher than the modified boiled cell method.

**Purity of the DNA (260/230 Ratio)**



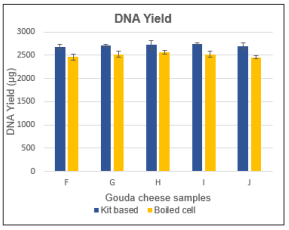

*Graph 03. The comparison of the DNA purity (260/230) of the extracted DNA from cheddar cheese samples via kit-based method and modified boiled cell method.*



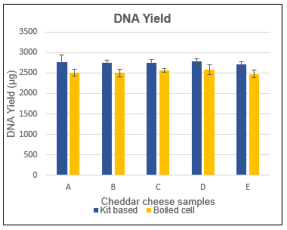

*Graph 04. The comparison of the DNA purity (260/230) of the extracted DNA from Gouda cheese samples via kit-based method and modified boiled cell method.*

The DNA purity, which was measured by the A260/230 ratio of extracted DNA samples from both methods, also indicated lower purity. However, the purity of the DNA samples extracted from kit-based method was higher compared to the boiled cell method.

**DNA Yields**




*Graph 05. The comparison of the DNA yields of the extracted DNA from cheddar cheese samples via kit-based method and boiled cell method.*




*Graph 06. The comparison of the DNA yields of the extracted from Gouda cheese samples via kit-based method and boiled cell method.*

All DNA samples extracted from kit-based method were indicated the higher DNA yields compared to the modified boiled cell method and DNA yields of kit-based method appeared within the same range.

**Statistical analysis of DNA yields**

*Table 07. Statistical analysis for the differences of DNA yields of cheddar cheese samples between two extraction methods by SPSS using one-way ANOVA (Significance level = 0.05).*

ANOVA

DNA yield

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 388513.200 | 1 | 388513.200 | 50.133 | .000 |
| Within Groups | 216988.800 | 28 | 7749.600 | | |
| Total | 605502.000 | 29 | | | |

*Table 08. Statistical analysis for the differences of DNA yields of gouda cheese samples between two extraction methods by SPSS using one-way ANOVA (Significance level = 0.05).*

ANOVA

DNA yield

| | Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Between Groups | 309676.800 | 1 | 309676.800 | 84.617 | .000 |
| Within Groups | 102472.500 | 28 | 3659.732 | | |
| Total | 412149.300 | 29 | | | |

The significance value (P=0.000) was less than 0.05 for both the cheddar and gouda cheese samples. Therefore, the differences in DNA yields between DNA samples, extracted via two methods was statistically significant.

**Identification of *Lactobacillus* by PCR – Modified boiled-cell method**

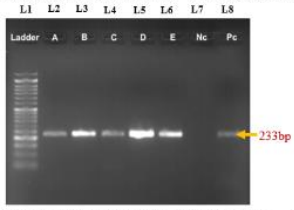



*Figure 05. 2% Agarose gel electrophoresis of PCR products amplified from extracted Lactobacillus DNA from cheddar cheese samples by modified Boiled cell method. Lane 1: 50bp ladder; Lane 2: Sample A; Lane 3: Sample B; Lane 4: Sample C; Lane 5: Sample D; Lane 6: Sample E; Lane 7: Negative Control; Lane 8: Positive Control.*

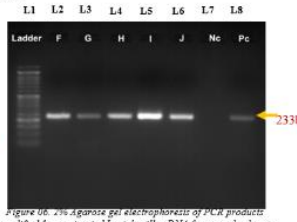



*Figure 06. 2% Agarose gel electrophoresis of PCR products amplified from extracted Lactobacillus DNA from gouda cheese samples by modified Boiled cell method. Lane 1: 50bp ladder; Lane 2: Sample F; Lane 3: Sample G; Lane 4: Sample H Lane 5: Sample I; Lane 6: Sample J; Lane 7: Negative Control; Lane 8: Positive Control.*

**Identification of *Lactobacillus* by PCR – Kit based method**

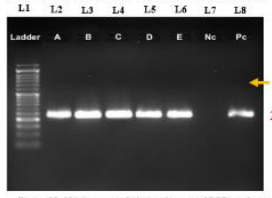



*Figure 07. 2% Agarose gel electrophoresis of PCR products amplified from extracted Lactobacillus DNA from cheddar cheese samples by kit-based method. Lane 1: 50bp ladder; Lane 2: Sample A; Lane 3: Sample B; Lane 4: Sample C; Lane 5: Sample D; Lane 6: Sample E; Lane 7: Negative Control; Lane 8: Positive Control.*

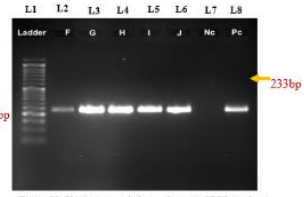



*Figure 08. 2% Agarose gel electrophoresis of PCR products amplified from extracted Lactobacillus DNA from gouda cheese samples by kit-based method. Lane 1: 50bp ladder; Lane 2: Sample F; Lane 3: Sample G; Lane 4: Sample H Lane 5: Sample I; Lane 6: Sample J; Lane 7: Negative Control; Lane 8: Positive Control.*

PCR products of band size 233bp were observed for DNA extracted from all samples by the two methods, coinciding with the positive control band and the negative control did not indicate any bands. Variations in the band intensities were observed.

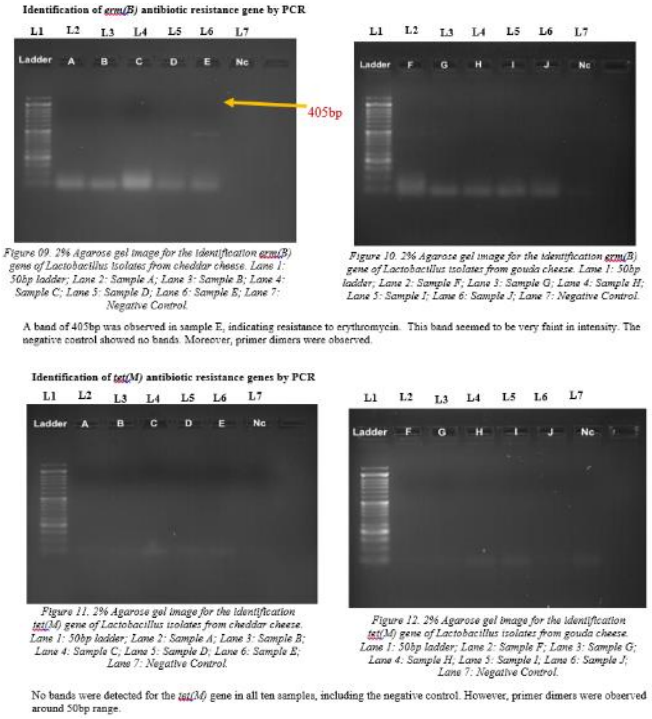



Figure 10. 2% Agarose gel electrophoresis of PCR products amplified from extracted Lactobacillus DNA from cheddar cheese samples by kit based method. Lane 1: 50bp ladder; Lane 2: Sample A; Lane 3: Sample B; Lane 4: Sample C; Lane 5: Sample D; Lane 6: Sample E; Lane 7: Negative Control; Lane 8: Positive Control.

## *DISCUSSION*

The consumption of probiotic foods, including curd, cheese, and yogurt promotes optimal health benefits and help to reduce risk of disease conditions (Ahmad et al., 2018). This study was performed to identify Lactobacillus, the commonest probiotic bacteria found in dairy products, from commercially available gouda and cheddar cheese samples and to evaluate the antibiotic resistance of these bacteria against selected antibiotics using molecular and microbiological techniques (Fijan, 2014). In order to identify Lactobacillus, phenotypical methods such as assessment of colony morphology and biochemical tests were carried out. Initially, the samples were cultured on MRS agar. The majority of the colonies observed in all ten samples were round, opaque, smooth, small and creamy white in color. This is consistent with the findings of Sharma and Goyal, (2015), Chakraborty and Bhowal, (2015) and Gad, Abdel-Hamid and Farag, (2014), who observed similar colony morphologies for various Lactobacillus species. Therefore, the colonies observed in this study could be of the genus Lactobacillus. The bacterial growth in each sample was differed which could be possibly occurred due to the different bacterial concentration added in each of the brands tested. Although, Lactobacillus specific MRS broth and MRS agar is selective medium for the growth of Lactobacilli, it does not suppress the growth of other lactic acid bacteria such as Streptococcus. This was evident since certain isolated colonies which subjected to the catalase test were indicated positive results, due to the bubble formation and gram staining of these colonies revealed gram-positive cocci. Moreover, MRS agar with cycloheximide could inhibit the accompanying growth of yeasts and fungi molds preventing contamination of the cultured sample (Yang et al., 2018). With repeated gram staining of various colonies, purple colored rods, in varied lengths were observed in all ten samples indicating the presence of gram-positive bacteria (Figure 02). Similarly, Umemoto, Sato and Kito, (1978) reported the presence of short and long rods in ripened cheddar cheese. Varying length of the rods is associated with different strains of Lactobacillus species. In the present study, the rods obtained in two different lengths could indicate the presence of different strains of Lactobacillus. The purity of the extracted DNA is a critical

factor in the sensitivity of the PCR as the inhibitory substances like proteins that are co-extracted with DNA could be present with the DNA samples. Those contaminants might be inhibited the PCR cycle resulting in false-negative results (Boesenberg- Smith, Pessarakli, and Wolk, 2012).

The A260/280 ratio can be used to judge DNA purity with regard to the protein contamination. High purity DNA have a A260/A280 of ~1.8 (Abdulamir et al., 2009) and the A260/A230 ratio, a secondary measure of DNA purity ranges in between 2.0-2.2 (Alrubaye et al., 2018). In this study, DNA extracted by the modified boiled-cell method and, the wizard protocol indicated A260/A280 purity values lower than the reference ranges confirming the presence of protein contaminants (Graph 1 and 2). Nevertheless, compared to the modified boiled cell method, the wizard protocol produced high DNA purity. Abed, (2013) was confirmed these findings by comparing four different extraction methods. This difference could be due to the addition of protein precipitation solution and RNase in the protocol which may have resulted in the removal of contaminants. Low purity of the DNA obtained via the boiled cell method could possibly be due to the lack of proper techniques for the removal of proteins in the boiled cell method (Abdulla, 2014). The mean A260/A230 for both the extraction methods were in between the values of 1.0 – 1.1, which was less than the standard values (Graph 3 and 4). This indicated the presence of polyphenols or organic compounds which absorb at 230 nm (Oliveira et al., 2014). Similarly, the low A260/A230 ratios could be caused due to EDTA contamination in both the methods as it absorbs at 230 nm (Lorenz, 2012). However, the DNA extracted via the two methods was not in the optimum purity. This could possibly occur due to the presence of contaminants during sample preparation since obtaining DNA with a higher purity is also dependent on sample quality and its preparation (Abed, 2013). However, PCR detection of Lactobacillus DNA was possible because of the dilution of DNA with TE buffer which could have diluted down the contaminating salts. Another reason could be the sensitivity of the PCR to low concentrations of DNA. According to graph 5 and 6, Wizard protocol resulted in the highest DNA yield in comparison to the modified boiled cell method. Statistical analysis shows that DNA yield significantly differed depending on the DNA extraction method used ($P<0.05$)(Table 07 and 08). It was further confirmed in the studies conducted by Abu Bakar et al., (2010) and Abdulla, (2014) indicating higher DNA yields in kit-based method. It is conceivable that a higher DNA yield for Wizard protocol can be attributed to the use of lysozyme in combination with EDTA as it provides more efficient lysis of the peptidoglycan layer in gram-positive bacteria (Alimolaei and Golchin, 2016). Hence, use of lysozyme in combination with EDTA and use of additional lysis step using nuclei lysis solution might have contributed higher DNA yield in samples extracted from the kit-based method compared to the boiled cell method (Abed, 2013). However, in the boiled cell method, DNA yield could be lower due to the availability of heat resistant strains found in fermented food products and the use of lysozyme alone (Without EDTA). Hence, cell wall lysis is less efficient (Abdulamir et al., 2010). Nevertheless, the yields and concentrations of extracted DNA from both methods were much higher compared to the findings of Abdulamir et al., (2010). This could be occurred due to several reasons such as the presence of a large number of Lactobacilli in the homogenized samples (high microbial biomass), errors in the DNA extraction

protocols or age and phase of growth of the microorganism (De et al., 2010).

Agarose gel images of boiled cell method (Figure 5 and 6) and wizard protocol (Figure 7 and 8) contain 233 bp bands for all 10 samples and the positive control which was the expected amplicons size for the primer confirming the presence of Lactobacillus DNA. Absence of band in the negative control indicates no contamination, no PCR inhibition and that the procedure has worked. No primer dimers and non-specific amplifications were observed in the gel images. Abed (2013) reported similar observations in his study. Successful amplification of the target gene indicated that the primers were specific to Lactobacillus genus. The faint bands seen in the images above could be due to loading errors which includes the insufficient amount of DNA loaded into the gel or preparation errors. Results obtained from PCR tallies with the biochemical tests done. PCR was used for the detection of tet(M) and erm(B) resistance genes in Lactobacillus positive samples. It was found that sample E harbors a 405-bp band for erm(B) gene (Figure 9) which correlates to the study conducted by Nawaz et al., (2011). Anisimova and Yarullina, (2018), has identified that both the erm(B) and erm(C) genes from Lactobacillus fermentum were responsible for erythromycin resistance. The rest of the samples did not contain any bands. No bands were detected for the tet(M) resistance gene (Figure 11 and 12). Nevertheless, presence of serval other genes or phenotypically resistant strains which may carries silent genes might be responsible for the tetracycline resistance in particular Lactobacillus strain (Gueimonde et al., 2013; Dec et al., 2017). The absence of bands in the negative control indicates no PCR contamination. Formation of primer-dimers can be minimized by proper primer designing and optimizing the protocol. However, Dec et al., (2017) reported that these genes can be passed on to the same or different bacterial species as they are carried on plasmids, transposons or integrons which can the play the role of a vector.

## *CONCLUSION*

This study aimed at isolating Lactobacillus from commercially available cheddar and gouda cheese samples. PCR amplification with genus-specific primers revealed the presence of Lactobacillus in all samples. The DNA yield obtained via the two extraction methods were statistically significant. Wizard protocol was found to be highly efficient compared to the modified boiled cell method as it resulted in a higher DNA yield and quality. The PCR assay for antibiotic resistance gene detection revealed an erm(B) gene in one sample. The presence of these genes in dairy products can pose a threat to the consumer as the resistance genes can be potentially transferable. Thus, it is necessary to include the screening of antibiotic resistance as a safety precaution prior to the use of Lactobacillus species as starter cultures or probiotics.

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# PANCREATIC $\beta$ CELL REGENERATION FROM HUMAN EMBRYONIC STEM CELLS AS A POSSIBLE THERAPY FOR TYPE 1 DIABETES

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## *ABSTRACT*

Type 1 diabetes (T1D) is an autoimmune disorder characterized by the destruction of functional insulin-producing pancreatic β-cells caused by autoreactive T cells. Two possible approaches for replenishing the β-cells are replacement by transplanting cadaveric islets or β cells derived from induced pluripotent stem cells (iPSC) or human embryonic stem cells (hESC) and induction of endogenous regeneration. As of today, the use of pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and induced PCSs (iPSCs) is one of the most promising therapeutic approaches. Production of glucose-sensitive, insulin-secreting beta cells derived from pluripotent hESC is an ideal cure for the treatment of diabetes. However, studies suggest the need to improve induction approaches as the production of insulin of these cells is lower than that of the endogenous pancreatic β-cells. The current review focuses on an overview of the advances in the generation of hESC-derived pancreatic β cells, generation of insulin-secreting islet-like clusters from hESCs in vitro and how the in vivo environment responds to glucose by the secretion of human C peptide and insulin when transplanted into animal models and the limitations and challenges of this therapy for the successful treatment of diabetes. Thereby, emphasizing the fact that further maturation of differentiated β-cells will be able to generate insulin-secreting cells for transplantation into patients with T1D as a potential therapy. Currently, the first phase 1/2 clinical trials with ESC-derived pancreatic progenitor cells are ongoing.

Keywords: Type 1 diabetes, pancreatic β cells, human embryonic stem cells

## *INTRODUCTION*

Diabetes mellitus is characterized by persistent hyperglycemia due to an impaired ability of the body to secrete or respond to insulin, or combination of both (Kharroubi and Darwish 2015). Diabetes mellitus can be classified as type 1 diabetes (T1D), type 2 diabetes (T2D), gestational diabetes (GDM) and other types by etiology and clinical presentation. Type 1 diabetes (juvenile diabetes or insulin-dependent diabetes) is an autoimmune disorder which is more common in children and teenagers (Goyal and Jialal, 2018). A study by Evans et al. in 2016 shows that among 415 million diabetic patients 10% are T1D patients (Figure 1). Pancreas plays the role in the energy consumption and metabolism. Less than 5% of total pancreatic mass represent the endocrine islets which synthesizes hormones (Zohu and Melton, 2018).

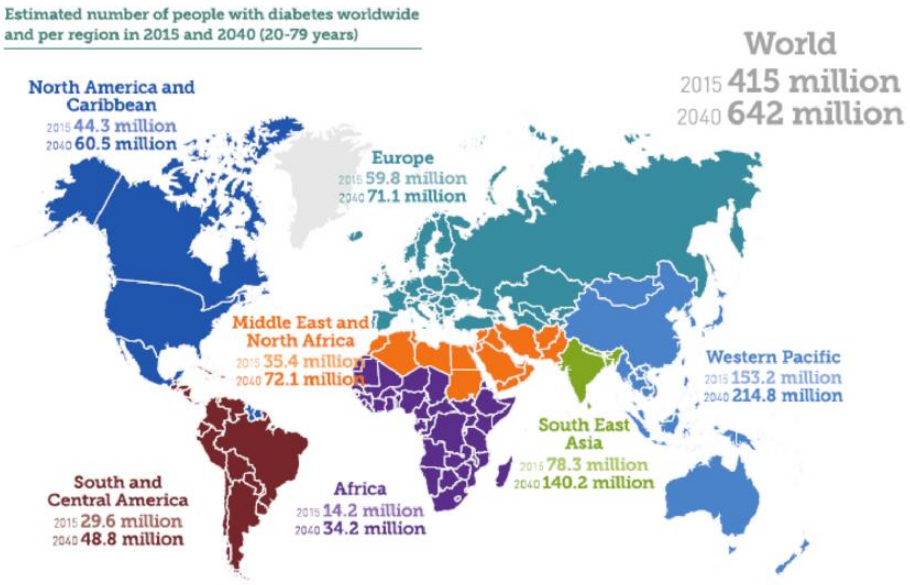



Figure 1. Worldwide prevalence of diabetes; 2015 and 2040 (Evans et al., 2016).

The autoimmune destruction of insulin producing β cells which is present in the islets of Langerhans will lead to hyperglycemic conditions. Continuous destruction or degeneration of beta cells causes T1D. Diagnosis of diabetes is done according to the criteria given in Table 1 below. Prolonged T1D disease may cause blindness, kidney failures, heart attacks, stroke and premature dying. Diabetes can be treated and the negative consequences can be avoided (Cito et al., 2018).

Table 1: Diagnosing Diabetes (adapted) (Kahanovitz, Sluss and Russell, 2018)

| Test | Results | Interpretation |
|---|---|---|
| HbA1c | ≥ 6.5% | Diabetes |
| | 5.7-6.4% | Impaired glucose tolerance |
| | ≤ 5.7% | Normal |
| Fasting Plasma Glucose | ≥ 126 mg/dL (7.0 mmol/L) | Diabetes |
| | 100–125 mg/dL | Impaired glucose tolerance |
| | ≤100 mg/dL | Normal |
| OGTT | ≥ 200 mg/dL (11.1 mmol/L) | Diabetes |
| Random plasma glucose | ≥ 200 mg/dL (11.1 mmol/L) | Diabetes |
| | 140–199 mg/dL | Impaired glucose tolerance1 |
| | ≤ 140 mg/dL | Normal |

There are number of approaches performed on T1D patients as shown in Table 2. The most significant therapeutic event in the history of type 1 diabetes was the discovery of exogenous insulin (Atkinson, Eisenbarth and Michels, 2014). Along with its therapeutical advancements it has been a major convenient therapy till date in most of the developing countries (Iqbal, Novodvorsky and Heller, 2018).

**Table 2:** Cons of β cell therapies (adapted) (Rodeman and Hatipoglu, 2018).

| Year | Strategy | Cons |
|---|---|---|
| 1922 | Exogenous insulin | • Short-term durability of the glucose sensor (about 1 week)<br>• Not a permanent treatment effect<br>• Long term administration |
| 1966 | Pancreas transplantation | • Donor shortage<br>• Life-long immunosuppressive treatment<br>• Open surgical procedure |
| 1977 | Islet transplantation | • Need to 2 to 4 donors per recipient<br>• Life-long immunosuppressive treatment<br>• Improvements are needed in harvesting islet cells and increased survival of transplanted donor cells<br>• Alloimmunity |

Despite the progress of these therapies, due to the above-mentioned problems, scientists have focused on stem cell research which may pave way into a realistic treatment for diabetes in the near future. Cells which have the potential for unlimited or prolonged self-renewal and to generate various other mature cell types are referred to as stem cells (Chagastelles and Nardi, 2011).

This literature review will focus on the in vitro pancreatic regeneration of β cells from ESCs which is a current ongoing clinical approach for T1D. Embryonic cells, first isolated from human embryos in 1998 are generally preferred as they are pluripotent and are able to differentiate into 200 cells representing all three germ layers; they are immortal in culture and will not senesce after several passages; and they maintain a typical chromosomal composition while adult stem cells have limited potency and differentiate into cell types of their own origin (Mahla, 2016; Chagastelles and Nardi, 2011).In this therapy, autologous embryonic cells are not used but cultured embryonic stem cells are prompted to differentiate in vitro, and thereby, the differentiated cells are implanted into patients. Currently, human embryonic stem cells, (hESCs) are the only stem cell population which are able to proliferate at a rate of >250 population doublings per year. These cells are capable of efficiently and rapidly differentiating to produce cells of all somatic lines by a series of defined developmental transitions (Kroon et al., 2008).

**Stem cell therapy to reverse diabetes**

Human embryonic stem cells (hESCs) are derived from human embryos that are a week old referred to as blastocysts. The lack of islet donors required for the treatment of T1D could be compensated by the development of hESC-derived insulin producing β-cells. Recent approaches in stem cell research have been successful in generating in vitro hESC-derived β cells. These cells have shown promising results when transplanted into animal models. But however, hESCs derived insulin-producing cells show deficiency in many functional characteristics compared with adult human pancreatic β cells. Below mentioned are a few recent clinical trials which have provided evidence to aid in the reversal of diabetes in animal models.

β- cell replacement in mice using hESC lines derived by somatic cell nuclear transfer

The process of nuclear transfer involves the removal of DNA from an immature egg cell and enucleating the donor oocyte with the genetic material derived from a body cell of the patient who is to undergo the embryonic stem cell transplant. Thus, embryonic stem cells are created by the removal of the inner cell mass of the pseudo fertilized egg once it reaches the blastocyst stage (Fortier, 2005). Nuclear transfer-derived human embryonic stem cells have recently been shown to produce insulin generating β-cells. Sui et al. in 2018 examined the ability of NT-ESs derived from a T1D patient to differentiate into functional β-cells and provide a source of autologous islets for replacement in diabetic mice. The differentiation capacity of 1018-NT-ES-derived β-cells was assessed by the result in the expression of endodermal genes as shown in Table 3.

**Table 3.** Gene expression of differentiated β-cells at each maturation stage (Sui *et al*., 2018).

| β- cell stage | β-cell marker | Percentage of gene expression |
|---|---|---|
| Pancreatic progenitor stage | SOX17 | ~95% |
| | FOXA2 | ~95% |
| | PDX1- | ~97% |
| | Co-expression of PDX1 and NKX6.1 | ~80% |
| β-cell stage (27 days of differentiation) | PDX1 | ~80% |
| | C-peptide | ~55% |

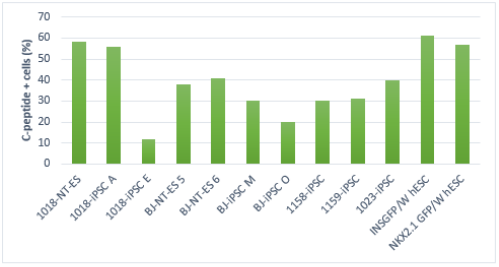
Moreover, various cell lines as shown in **Table 4**, were used to compare the differentiation potential of β-cells into C-peptide–positive cells.

**Table 4:** Information on hESC lines and iPSC lines derived from type 1 diabetic patients and healthy subjects (Sui *et al*., 2018)

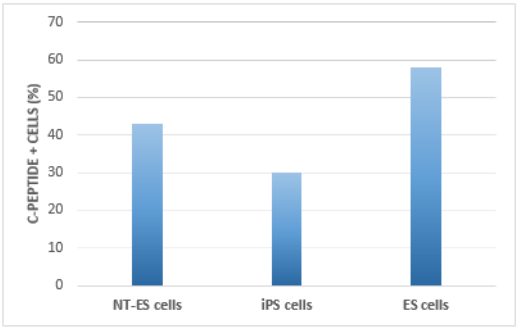
| ID | Diagnosis | Stem cell line ID |
|---|---|---|
| 1018 | T1D | 1018-NT-ES<br>1018-iPSC A<br>1018-iPSC E |
| BJ | Healthy control | BJ-NT-ES 5<br>BJ-NT-ES 6<br>BJ-iPSC M<br>BJ-iPSC O |
| 1158 | T1D | 1158-iPSC |
| 1159 | Healthy control | 1159-iPSC |
| 1023 | Healthy control | 1023-iPSC |
| $INS^{GFP/W}$ hESC | Not applicable | $INS^{GFP/W}$ hESC |
| NKX2.1 $^{GFP/W}$ hESC | Not applicable | NKX2.1 $^{GFP/W}$ hESC |

It was noticed that the number of C–peptide positive cells obtained from isogenic 1018-NT-ES was significantly higher than 1018-iPSC E cell line. The number of C-peptide cells were also reduced in the BJ- human induced

pluripotent stem cell line- O compared with that of the isogenic BJ-nuclear transfer embryonic stem cell line. On average, iPSC cell lines showed a reduced C-peptide differential potential (Graph 1 and 2).




***Graph 1:*** Comparison of the derivation of C-peptide positive cells from various cell lines (adapted) (Sui *et al.*, 2018).




***Graph 2:*** Overall comparison of NT- ES, iPSC and ES cell lines (adapted) (Sui *et al.*, 2018).

Upon transplantation, 1018-NT-β-cells were able to cause the reversal of diabetes in animal models by secreting insulin during high glucose concentrations in blood, and decrease insulin secretion during low levels of blood glucose. These results provide evidence of how suitable the NT-ES derived β-cells are for the substitution of islets in patients with T1D. Further studies are required to determine the molecular basis for the differences in efficiency of the in vitro differentiated cells compared with the human pancreatic β-cells.

Influence of the in vivo environment upon transplantation of differentiated β-cells.

The lack of islet donors required for the treatment of diabetes could be compensated by the generation of hESC-derived β-cells to restore normoglycemia in diabetic patients. No complete physiologically active β-cells could be generated in vitro although various protocols have been established for the derivation of pancreatic progenitors from hESCs. Sui et al., (2013) conducted a study to examine the in vivo growth of these cells obtained from hESCs following transplantation in the fat pad or subcutaneous site. Twenty-one (21) mice in total were implanted with PDX1-( pancreatic and duodenal homeobox 1) positive pancreatic endoderm (PPP) cells either into the epididymal fat pad or the dorsal subcutaneous space. It was observed that 2 weeks after implantation only few PDX1+ cells remained in the fat pad site. Subcutaneous grafts in 6 weeks' time continued to express PDX1 at all analysed time points and after a period of 6 weeks exhibited co-expression of PDX1 and homeobox protein NKX6.1. Furthermore, between 6 and 12-weeks post transplantation they generated NGN3+ cells as well as some C-peptide positive cells. In addition, 6 weeks post implantation cartilage tissue developed in the fat pad from contaminating MSCs present in the graft, but not in the subcutaneous space.

According to this study, it tends to be presumed that the in vivo microenvironment contributes in the further differentiation of the implanted PDX1+ β cells. As of now, it is not yet known as to why the transplanted hESC-derived pancreatic β-cells responded differently at the two locations. Probably, it is because the subcutaneous site provides the required factors for the survival and proliferation of the cells that the fat pad does not. A similar study was

conducted by Matveyenko et al. in 2010 where hESC-derived PDX1+ / NGN3+ (Neurogenin-3) cells was transplanted into the epididymal fat pad in a total of 15 nude rats. However, after a period of 20 weeks post- transplantation, a small amount of differentiated β cells were detected in only half of the grafts implanted. The failure of the development of the pancreatic endoderm into remarkable physiologically active glucose-sensitive insulin secreting cells when implanted into the fat pad in the nude rats as implanted previously in mice can be due to various possible causes. It is unlikely to be due to technical issues of implantation or insufficient cells implanted. It is conceivable that the nude rat model is a less accommodating host than the nude mouse model post-transplantation of the PDX1+ pancreatic endoderm cells. Additionally, Eshpeter et al., (2008) and Phillips et al., (2008) could not identify any insulin secreting β-cells until 6 weeks post transplantation, and the transplanted diabetic animals did not show any significant decrease in the levels of blood glucose. It was assumed that different results could be obtained if the transplantation time was prolonged in order to allow further differentiation of the NGN3+ progenitors into mature insulin producing β cells as Sui et al., (2013) detected insulin producing cells 12 weeks post transplantation. Analysing the data from the above-mentioned studies supports the beneficial effect of an in vivo environment. However, unfortunately the impact of such as environment is not known in precise.

**hESC-derived β cells restores blood glucose levels through intra-spleen migration.**

Extensive investigation of the implantation of insulin-producing cells derived from embryonic stem cells (ESC) has been taken place in search of a cure for T1D. However, the mechanism of the transplanted cells in vivo remains uncertain and needs further investigation in diabetic animal models. There have been significant shortcomings such as scarcity of animal models at scheduled timings, lack of studies on a longitudinal basis in the same organism and constrained usage of them in clinical studies in obtaining facts regarding the in vivo behaviour of transplanted pancreatic like β-cells in diabetic mice via histological means. Thus, the urgent need for a non-invasive approach for assessing cell distribution and migration for both human and animal trials in stem cell-based studies arose over time. Ren et al., (2014) investigated the site and movement of insulin-producing cells labelled with super-paramagnetic iron oxide (SPIO) through dynamic MRI (magnetic resonance imaging) as it is an appropriate tool to characterize anatomic information in a non-invasive way. After transplantation of SPIO labelled insulin generating cells into the renal sub-capsular area of diabetic mice, hypo-intense signals appeared in the spleen after one week of transplantation and turned out to be progressively evident by the fourth week and remained as such over the entire study period while the MRI signal intensity under kidney sub-capsules declined to nearly 60% after 6 weeks of cell delivery (Figure 2).

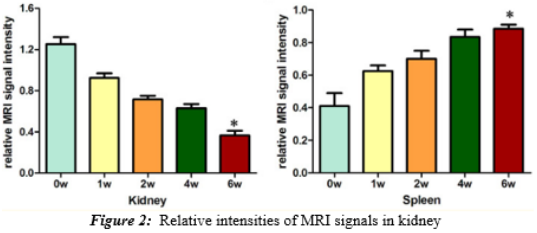



***Figure 2:*** Relative intensities of MRI signals in kidney and spleen over a 6-week period (Ren *et al.*, 2014).

Furthermore, this study compared the glucose levels in blood and the rate of survival of cells when transplanted into renal sub-capsules to those of cells transplanted into the spleen. Each transplantation group consisting of a

sample number of 10 diabetic mice. Results demonstrated that blood glucose control was reached in spleen by the fourth day post- transplantation, ahead of the transplantation in the kidney sub-capsules (Figure 3). But, no statistical significance (62.5%) was detected in the rate of survival of the cells in both the spleen and kidney transplantation group by the end of the study.

As demonstrated by the results, it is not through a direct pathway that the migration of C-peptide positive cells takes place from the kidney into spleen but via the circulation of blood. However, the molecular mechanisms involved in the migration of the grafted cell remains doubtful. The reason for cell migration into the spleen is assumed to be the good vascular supply and the oxygen-rich microenvironment for the islets which the kidney sub-capsules unfortunately lacks. Usage of MRI allows repeated observations in the same living organism, which would expand our comprehension of in vivo stem cell behaviour post-transplantation and could help in the advancement of therapies for the treatment of T1D diabetes. But results have been varied in a study conducted by Bruin et al., (2013) where he transplanted hESCs derived pancreatic progenitor cells under the kidney capsules and found it out to be a suitable location for the transplantation of cells in rodent models. However, a major limitation of that study was the cell formation resembling bone and cartilage. Taken together, these studies confirm that under a range of in vivo conditions pancreatic progenitor cells derived from hESC are capable of treating T1D.

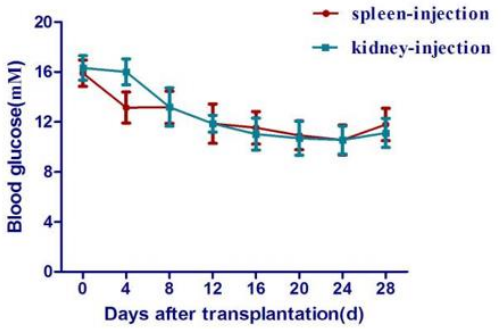


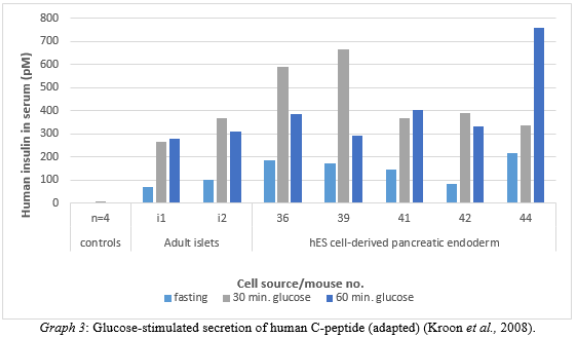

***Figure 3***: Blood glucose levels in the groups transplanted into kidney sub-capsules (n=10) (blue lines) or into spleen (n=10) (red lines) (adapted) (Ren *et al.*, 2014).

Generation of glucose-responsive insulin-secreting β-cells by hESCs derived pancreatic endoderm

Production of functional insulin secreting β-cells that respond to glucose would aid in the advancement of a cell-based therapy for T1D. Jiang et al., (2007), developed a 36 day protocol where hESCs were differentiated into islet like clusters with characteristics like those of pancreatic β cells in the presence of signalling molecules and specific growth factors in vitro. It was observed that the hESC-derived islet like clusters exhibited multiple β-cell specific genes as mentioned in Table 4. By day 36, Pdx1+ cell clusters differentiated further and in response to the stimulation of glucose, cells secreted pancreatic hormones such as insulin, glucagon and somatostatin. To add to this study, Kroon et al. in 2008 showed that pancreatic endoderm derived from human embryonic stem (hES) cells efficiently generate endocrine cells that respond to glucose upon transplantation into in mice. Upon glucose administration in implanted mice, C-peptide and human insulin were detected at similar levels to those of mice implanted with approximately three thousand adult human pancreatic β cells. Moreover, the insulin-expressing cells post engraftment exhibited many characteristics of physiologically active β-cells, including

expression of mature endocrine secretory granules, transcription factors belonging to the beta- islets and appropriate processing of proinsulin. The differentiated pancreatic β cell clusters were distinguished by the co-expression of FOXA2, PDX1 and HNF6; NKX6-1 was also expressed by the majority. The differentiated β- cells were implanted into both epididymal fat pads in 105 male immunodeficient mice.

Thirty-sixty days post transplantation the team assessed if the human embryonic stem cell–derived pancreatic endoderm produced in vitro can generate functionally active β-cells in vivo. This was analysed by the detection for serum levels of human C-peptide at fasting, 30 minutes and 60 minutes after intraperitoneal glucose administration at the indicated post-engraftment times. One-month post implantation reduces serum C-peptide levels were detected. During the next 2 months, there was a rapid rise in human C-peptide response in most mice when measured during both FPG and stimulated plasma glucose. Three months post-engraftment, fasting levels stabilized whereas the stimulated glucose human C-peptide serum levels continued to rise (Graph 3).




*Graph 3*: Glucose-stimulated secretion of human C-peptide (adapted) (Kroon *et al.*, 2008).

Recently, Rezania et al. in 2014, detailed a multiple stage protocol that led to efficient embryonic stem cell differentiation into glucose-responsive insulin-producing β cells in vitro. These cells secreted insulin upon glucose-stimulation in vitro similar to that of human pancreatic β cells and reversed diabetes in STZ -induced diabetic mice within a timeframe of 3 months post-transplantation.

Together, this information provides compelling proof that hESC–derived pancreatic endoderm cells are able to differentiate into glucose-responsive, insulin-secreting β cells and aid in the reversal of diabetes in animal models. By all measures examined, these islets are physiologically very similar to pancreatic islets of humans, providing definite evidence that hES cell–derived pancreatic endoderm cells may benefit as an alternative source of islets for cell-replacement therapies in T1D patients. Further research is needed to improve the understanding of how the in vivo environment of an animal model influences cell migration and distribution, if addition of autologous vascularized cells improves engrafted PSC-derived β cells function against auto/alloimmune reaction or to determine whether or not, the non-insulin-producing cells are beneficial for hESC derived β-cell differentiation. Studies need to be conducted on the safety of the transplantation as different stages in cell progression has a significance involvement in the safety issue, since it is presumed that cells at the progenitor stage have a certain degree of residual plasticity and a greater proliferative capacity which should be significantly reduced in the differentiated mature cells, making these the safest. Furthermore, studies need to validate if an in vitro or in vivo-differentiated iPSC therapeutic approach is better due to the possibility of teratoma formation.

In the past few couple of years a lot of exertion has been directed to the encapsulation of cells in macro-devices

which are considered safe, biocompatible and permselective. Macro-encapsulation devices have been developed to provide protection to cells against immune reactions and to allow the removal of the device in case of the formation of tumours. ViaCyte, in 2014 conducted the first phase I/II human clinical trial assessing the safety, long-term effects and efficiency of a PEC-Direct product, where pancreatic progenitors derived from human ESC (named PEC-01) were encapsulated in a device and implanted in a small number of patients diagnosed with T1D. Moreover, in the year 2017, ViaCyte's PEC-Direct product has been granted approval for clinical testing by the U.S. Food and Drug Administration (FDA). Recently, ViaCyte reported good tissue coordination and increased vascularity in two patients of the ongoing clinical trial and the outcomes of the trial are expected to be revealed soon. Another device, the CPS which is a macro-device non harmful to the living tissues created to be transplanted into the subcutaneous site in T1D patients received permission for phase I/II of the clinical trials from the FDA (Cito et al., 2018). Table 5 summarizes the clinical trials which are in progress from ViaCyte. The results of the human clinical trials in progress is expected to generate valuable data and will tell us if this is the therapy for the cure of diabetes in future.

**Table 5:** Clinical studies of ViaCyte testing safety, tolerability and efficacy of ESC-derived PEC-01 cells encapsulated into a macro-device (Encaptra cell delivery system) (Cito *et al.*, 2018).

| Identifier | Name | Cell product | Study type | Conditions | Status |
|---|---|---|---|---|---|
| Nbib2239354 | A safety, tolerability and efficacy study of VC-01 combination product in subjects with type 1 diabetes mellitus | VC-01 combination product (PEC-Encap) | Phase 1/2 | Type 1 diabetes mellitus, no immunosuppression | - |
| Nbib3162926 | A safety and tolerability study of VC-02 combination product in subjects with type 1 diabetes mellitus | VC-02 combination product (PEC-Direct) | Phase 1 | Type 1 diabetes mellitus, with immunosuppression | Active, not recruiting |
| Nbib3163511 | A safety, tolerability and efficacy study of VC-02 combination product in subjects with type 1 diabetes mellitus and hypoglycemia unawareness | VC-02 combination product (PEC-Direct) | Phase 1/2 | Type 1 diabetes mellitus with hypoglycemia, with immunosuppression | Active, recruiting |

## *CONCLUSION*

Diabetes mellitus is a severe health problem amongst the public, and its prevalence is increasing world-wide. The available treatments such as exogenous insulin and transplantation of purified human cadaveric islets can neither cure nor completely control the complications of this disease, which results in the loss of a great number of lives. Thus, there is an urge for the development of new treatments that provide adequate blood glucose control to minimize long-term diabetic complications. Hence, the need for alternative β cell sources of arose such as differentiation of hESCs into functionally active insulin-secreting β cells, to treat the increasing number of diabetic patients. Data from the studies used in this review supports the beneficial effect of an in vivo environment while its influence remains unclear. Moreover, effective methodologies are not yet available to protect hESC derived endoderm implants from teratoma formation or immune rejection arising from the implant. This review strengthens the fact that hESCs derived pancreatic like β islets is the most attested new source for the replacement of cells at the moment in T1D patients. In the years to come, the field of embryonic stem cell therapy will be able to transform strategies restricted to a small number of patients into a beneficial therapy readily available for a large cohort with the help of developing technology.

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# IDENTIFICATION OF STREPTOCOCCUS IN TABLE OLIVE SAMPLES AND ANALYSIS OF THEIR ANTIBIOTIC RESISTANCE

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## *ABSTRACT*

Fermented olives are a rich source of nutritional compounds and probiotics. Probiotics are defined as living bacteria that confer health benefits to the host when administered in adequate amounts. The purpose of this study was to identify the genus of Streptococcus in fermented olives samples and analyze their antibiotic resistance. The process was carried out by culturing 5 samples in MRS agar followed by biochemical tests to identify pure Streptococcus colonies and then sub culturing was performed. Afterwards DNA extraction was carried out by boil cell and kit based extractions method. The DNA yield and purity were compared between the two methods. PCR was performed using genus specific primers to identify Streptococcus. In addition to that, the resistance gene for tet(M) and erm(B) were detected by PCR and visualized by gel electrophoresis. Out of 5 samples, 4 samples had proper growth in MRS agar. After extraction when comparing DNA yield between the two methods were statistically significant (p-value $< 0.05$). The highest yield was observed by boil cell method. Out of 4 samples, 2 were positive for streptococci in PCR identification. Sample 2 was identified to contain erm(B) and tet(M) was not found. Some probiotics have been reported to harbour antibiotic resistance genes which can be transferred to pathogens in the gut. Therefore probiotic bacteria used in the food industry should be screened for resistance genes in order to ensure safety of the consumers.

Keywords: Streptococcus, Probiotics, Antibiotic Resistance

## *INTRODUCTION*

Fermented olives, also known as table olives are consumed worldwide, especially in the Mediterranean countries. These olives has a great impact on the worldwide economy, since they have high nutritional content including vitamins, fibers, unsaturated fatty acids, amino acids and antioxidants (Moumita et al., 2018). These nutrients make olives a significant functional food in the meal. Moreover, the fermentation along with preservation eventually enhances the technological plus nutritional characteristics with the health benefits (Guantario et al., 2018).

The most frequently used and important functional food compounds are probiotics. These are various living bacteria that, when ingested in sufficient amounts, presents advantages on the host health. It is a part of the microbiota due to their beneficial health effects and hence it has been used for centuries (Xiao et al., 2017). Probiotic foods contain live microbes in an adequate amount to reach the intestine (Bron and Kleerebezem, 2018). They provide the balance of host intestinal microflora by the stimulation of the helpful microorganisms and the reduction of harmful bacteria (Varcamonti et al.,

2006). To express the health benefits, probiotic should contain at least 106–107 colony forming units/g in the food which is consumed.

There are number of requirements to select particular probiotic strain, which is safe for the host and it is important to assure safety, even in the middle of bacteria that are in generally recognized as safe (GRAS). Lactic acid bacteria (LAB) have a significant attention as probiotics over past few years. Mostly, LAB consists of rod-shaped microorganisms such as Lactobacilli, Bifidobacterium and cocci such as Streptococci, Lactococci, (Ben-Yahia et al., 2012) and Enterococci. These bacteria exist in the human gut flora and can also be taken orally as they are available in food products such as fermented vegetables and dairy products. The most important properties of probiotic include: gastric and bile acid resistance, adherence to mucus and/or human epithelial cells and cell lines, antimicrobial activity against potentially pathogenic bacteria or fungi (Pu et al., 2017), bile salt hydrolase activity and the enhancing viability (Hummel et al.,2006). It should be stable during processing and storage and predominantly it should be safe to consume. Prevention of diarrhea, constipation, improvement of the anti-bacterial activity, changes in bile salt conjugation and anti-inflammation are several health benefits associated with probiotics (Schmitter et al., 2018)

Streptococcus is a gram-positive spherical bacteria that belongs to family Streptococcaceae, order Lactobacillus also known as Lactic acid bacteria, and belongs to phylum Firmicutes. Under microscopic identification of Streptococcus spp. can grow as pairs or chains that may appear bent or twisted since cell division in streptococcus arises along a single axis therefore the bacteria is able to form chains and pairs (Evivie et al., 2017). Most of the streptococci are oxidase-negative and catalase- negative and many are facultative anaerobes who are capable of growing under both aerobic and anaerobic conditions. Streptococcus thermophilus is one most important probiotic strains and also known as Streptococcus salivarius (Fontaine et al., 2009). Streptococcus thermophilus is a thermophilic LAB with key importance for the dairy production where strains of the species are generally used in manufacturing many cheeses varieties and yoghurt (Flórez and Mayo, 2017).

Antibiotic resistance (ABR) has become a most vital threat to the general communities' health (Devirgiliis, Barile and Perozzi, 2011). ABR bacteria can cause diseases which lead to dangerous infections and sometimes even death. It has become a difficult task to kill ABR bacteria and more it is costly to treat as well. There are few ways that bacteria can develop and acquire resistance to antibiotics; "neutralize" the antibiotic by altering it in a way that makes it harmless (De Vries et al., 2009), pumping antibiotics to the outer surface of the bacteria before it can do any harm (Ishihara et al., 2013), changing their outer structure by blocking binding sites and etc. After being exposed to antibiotics, sometimes even one bacteria can survive as it finds a way to counter attack the antibiotic and becomes resistant giving rise to a number of bacteria which can replace the dead bacteria (Gueimonde et al., 2013). After exposure by the selective pressure obtained from antibiotics the bacteria can become resistant or it can become resistant during a mutation of their genetic material (Villavicencio et al., 2018; Tsuyuki et al., 2017; Rizzotti et al., 2009) as shown in Figure 1.

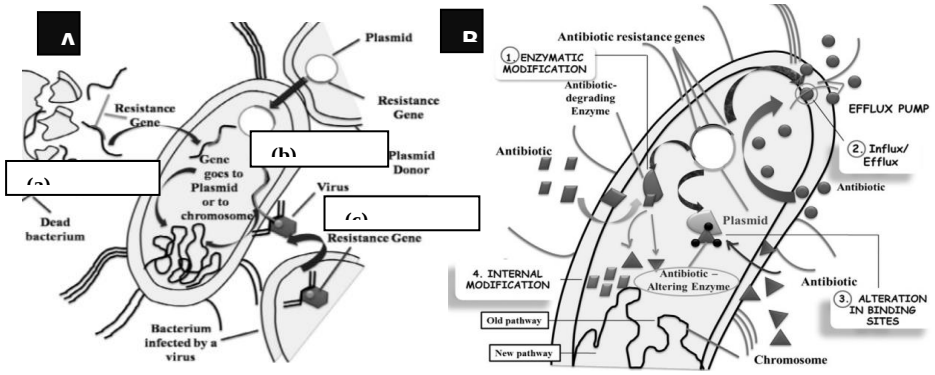


Figure 1. Mechanisms of antibiotic resistance in probiotics. (A) Intrinsic antibiotic resistance 1) efflux pumps, 2) antibiotic degrading enzyme, 3) antibiotic altering enzyme and 4) Inner change (B) Acquired antibiotic resistance (a) transformation, (b) conjugation, and (c) transduction (Sharma et al., 2014).

Antibiotic resistance of streptococcus can be determined by minimal inhibitory concentration (MIC) and disk diffusion assay (Brenciani et al., 2103). In addition, the presence of resistance gene(s) can be evaluated by molecular methods identification of streptococcus spp. using PCR amplification which is considered as a gold standard method and to identify antibiotic resistance the most frequent used resistance genes are tet(M) and erm(B). Molecular methods are conventional, specific and efficient over culturing methods, other than that the consumed time is lesser than the culturing methods. Antibiotic resistance has become an urgent threat to public health and despite the health benefits some probiotics have reported to harbor resistance and these can be transferred to other bacteria. The Purpose of this study was to identify probiotic genus of Streptococcus in fermented olives samples and analyze their antibiotic resistance. Therefore, it can be prevented and probiotics can be safely engineered to commercialize and to be used for next generation.

## *METHODOLOGY*

**Sample preparation**

Five international brand of fermented olives were purchased from local market. Five separate beakers were taken and labeled from 1 to 5. Followed by transferring of the different samples (one olive and 5 ml of brine samples) into their respective beakers in equal amounts.

Culturing of the Streptococcus sample in MRS agar

Aseptic conditions were followed throughout the procedure. Using a sterile inoculation loop, the sample (crashed olive and brine mixture) was picked. It was streaked on MRS media using the four quadrant streak plate method. After streaking, all five petri plates were parafilmed and placed in the incubator. It was incubated in 37°Cfor 2 days.

**Biochemical Tests to identify streptococcus**

Gram staining of the cultivated colonies from the MRS agar

Initially, the bacterial smear was prepared with the specific colony picked and placed on a clean glass slide and was allowed to air dry and fixed by flaming. Then a drop of crystal violet was added and was allowed to dry for 30 - 60 seconds. The excess, was washed out. Thereafter, a drop of Grams iodine was added to the slide and it was allowed to air dry for 1 – 2 minutes. The excess amount

of iodine was washed out. Grams decolourizer (10 – 30 secs) was added to wash off excess stain followed by the addition of safranin (10 – 30 secs). The slide was washed with water and allowed to dry. A cover slip was placed and observed under the microscope (10x, 40x and but most importantly 100x (oil immersion)).

**Catalase test to identify anaerobic bacteria**

Aseptic conditions were followed throughout the procedure. Using a sterile inoculation loop, the colony sample from the previously cultured plate was picked and mixed with a water drop which was on the slide and a drop of H2O2 was added.

Sub culture of the Streptococcus pure colonies in MRS agar

Aseptic conditions were followed throughout the procedure. Using a sterile inoculation loop, the colony sample from the previously cultured plate was picked. It was streaked on MRS media using the four quadrant streak plate method. After streaking, all four petri plates were parafilmed and placed in the incubator. It was incubated in 37°C and 45oC for 24 hours.

**DNA extraction of the cultivated colonies from the MRS agar**

Heat shock DNA extraction method

Aseptic conditions were followed throughout the procedure. A 2 ml of autoclaved distilled water was added to each labelled falcon tube (1-4) followed by an inoculation of sufficient amount of the selected colonies into tube. The falcon tubes were then centrifuged at 4000rpm for 20 minutes. The supernatant was discarded and to each tube 500µl of TE was added to each tube and vortexed briefly. Thereafter, the tubes were heated at 100°C for 20 minutes and immediately cooled at -20°C for 20 minutes. Then the tubes were centrifuged at 4000rpm for 10 minutes. Finally the pellet formed was discarded and the supernatant was transferred to the Eppendorf tubes and stored at -5°C.

Promega kit based extraction method

The DNA extraction procedure was carried out by promega kit method. In the initial step bacterial colonies were scraped and mixed with it 2 ml of autoclaved distilled water which was in eppendorf tube and centrifuge at 7000rpm for 4 minutes and water was removed.it was suspended in 480µl of 50mM EDTA and 120µl of lytic enzyme was added was added. Then incubated at 37oC for 30-60 Minutes, after that centrifuged the tubes in 7000rpm for 4 minutes add supernatant was removed. Then 600µl of nuclei lysis solution was added into the pallet contained eppendorf tube and gently mixed with pipette. Then the samples were incubated in the water bath at 800C for 5minutes. After that samples were let to cool down at room temperature. Here after 3µl of RNase solution was added and mixed. In the next step, samples were incubated again in the water bath at 370C for 45 minutes and kept in the room temperature to cool down. After this the samples were transferred into the labelled eppendorfs tubes using pipettes. 200µl of protein precipitation solution was added, and then samples were vortexed for 5-10 seconds. Then placed into ice and incubated for 5 minutes and then samples were centrifuged at 7,000 rpm for 6 minutes. Next the supernant of the samples were transferred in to the clean labelled eppendoff tubes which consisted with 600µl of isoproponal, and samples were mixed well with tapping and subsequently the samples were centrifuged at 7,000 rpm for 4 minutes. Here after the supernant of the samples were removed and 600µl of 70% ethanol was added into the samples at room temperature, consequently samples were mixed well by tapping. And then the centrifugation procedure at 7,000rpm for 4 minutes. Following that ethanol was aspirated via discarding form the samples, and afterwards the samples were placed to

air dry for 15-20 minutes. Once this procedure done, 100µl of DNA Rehydration solution was added into the samples to rehydrate the DNA pallet and subsequently the samples were stored at 40C temperature for further analysis.

Quantification of DNA from bacterial DNA isolated from Boiled cell and Kit based extraction methods using spectrophotometer

DNA quantification for the two extraction methods was performed in a spectrophotometer to calculate the DNA concentration and DNA yield for each sample.

Readings were taken in triplicates at 230, 260 and 280nm wavelengths and the mean was calculated. These readings were taken for each of the 5 samples (Sample 1 – 5).initially Absorbance of TE was measured since it was been used as the blank.

Following the calculation of the DNA concentration and yield (Refer appendix B), the ratios (260/280) and (260/230) were calculated for both extraction methods to evaluate the purity of DNA. Equations used are mentioned below and these were adapted from a study conducted by Abdulamir et al., (2010):

Dilalution factor=(TE Used as Blank(3000µl)+Amount of DNA sample added(30µl))/(Amount of DNA sample added (30µl))

DNA concentration (n⟦gµl⟧^(-1))=(A260 in OD units)×⟦50µgml⟧^(-1)×Dialution Factor

DNA yield (µg)=DNA concentration(⟦µgµl⟧^(-1) )×Amount of DNA kept as a stock

Statistical analysis of the DNA yield and its comparison with the sample and extraction method

Statistical analysis was performed using one way ANOVA analysis in SPSS Statistics 21 software. This was done to find if there is any significance (p value <0.05) or there is no significance (p value >0.05) between the DNA yield when comparing it to the sample and the extraction method.

**Identification of Streptococcus by Polymerase Chain Reaction (PCR)**

Streptococcus was identified using genus specific primers (Table 1), Volumes and reagents used are included in Table 2. PCR cyclic conditions are given in Table 5 (Prabhu et al., 2012) PCR was performed using DNA extracts from both extraction methods.

*Table 1. Streptococcus genus specific primer sequences and product size*

| Target Organism | Primer Set | Sequence (5' to 3') | Product size | Reference |
|---|---|---|---|---|
| Streptococcus genus | Forward primer | CAA CTT GAC GAA GGT CCT GCA | 110 | Prabhu *et al.*, (2012) |
| | Reverse primer | TGG GTT GAT TGA ACC TGG TTT A | | |

*Table 2. Components of the PCR reaction mixture and their specific working concentrations*

| Reagents | Stock Conc. | Working Conc. | Volume for one reaction (µl) | Volume for 6 reactions (µl) |
|---|---|---|---|---|
| PCR buffer | 5X | 1X | 5 | 30 |
| dNTP | 10Mm | 0.2 Mm | 0.5 | 3.0 |
| Forward Primer | 2 µM | 0.2 µM | 2.5 | 15 |
| Reverse Primer | 2 µM | 0.2 µM | 2.5 | 15 |
| MgCl2 | 25 mM | 1.5 mM | 1.5 | 9.0 |
| Taq Polymerase | 5 U/ µl | 0.05 U/ µl | 0.25 | 1.5 |
| DNA | - | - | 100 ng/µl | - |
| Autoclaved distilled H20 | - | - | - | - |
| Total volume | | | 25 | 73.5 |

The amount of DNA and autoclaved distilled water added for each sample was given in Table 3 and Table 4 since the DNA amount added vary from sample to sample.

Initially the PCR mastermix was prepared consisting of PCR buffer, dNTP, Forward primer, Reverse primer, MgCl2 and Taq polymerase. The mastermix was prepared for 7 reactions (5 samples + negative control + extra reaction). Followed by the aliquotion of the PCR master mix (12.25 µl) to each tube. Amount of DNA was calculated by below mentioned equations:

Amount of DNA = DNA working conc. (100ng/ µl) / DNA concentration

Amount of Water = Total volume (25 µl) – (12.25 + Amount of DNA)

Table 3. Amount of DNA and Water need for each sample for the PCR reaction for boiled cell method

| Sample | Amount of PCR Mater mix added(µl) | Amount of DNA added(µl) | Amount of water added(µl) |
|---|---|---|---|
| Negative control | 12.25 | - | 12.75 |
| Sample 1 | 12.25 | 0.7 | 12.05 |
| Sample 2 | 12.25 | 2.0 | 10.75 |
| Sample 3 | 12.25 | 0.3 | 12.45 |
| Sample 4 | 12.25 | 0.3 | 12.45 |

*Table 4. Amount of DNA and Water need for each sample for the PCR reaction for Kit Based extraction method*

| Sample | Amount of PCR Mater mix added(µl) | Amount of DNA added(µl) | Amount of water added(µl) |
|---|---|---|---|
| Negative control | 12.25 | - | 12.75 |
| Sample 1 | 12.25 | 2.5 | 10.25 |
| Sample 2 | 12.25 | 2.1 | 10.65 |
| Sample 3 | 12.25 | 4.3 | 8.45 |
| Sample 4 | 12.25 | 8.5 | 4.25 |

PCR cyclic parameters for Heat Shock and Kit based extraction methods

Table 5. PCR cyclic parameters for Heat Shock and kit based extraction PCR procedure

| Primer Set | Sequence (5' to 3') | Product size | Reference |
|---|---|---|---|
| tet(M) forward | GGTGAACATCATAGACACGC | 401 | (Gad, Abdel-Hamid and Farag, 2014). |
| tet(M) reverse | CTTGTTCGAGTTCCAATGC | | |
| erm(B) forward | CATTTAACGACGAAACTGGC | 405 | |
| erm(B) reverse | GGAACATCTGTGGTATGGCG | | |

| Step | Temperature (°C) | Time/min | Cycles |
|---|---|---|---|
| Initial denaturation | 95 | 5 | |
| Denaturation | 95 | 1 | 35 |
| Annealing | 49.5 | 1 | |
| Extension | 72 | 2 | |
| Final Extension | 72 | 12 | |
| Final hold | 4 | | |

PCR for the identification of resistance for tetracycline and erythromycin towards Streptococcus isolates

PCR was performed for the Streptococcus positive samples using both tet(M) (tetracycline) and erm(B) (erythromycin) resistant genes specific primers separately according to Table 6. The same PCR components (Table 2.) were used in both of these PCR procedures but the number of reactions for each including the amount of DNA and water added were calculate and according to Table 7 for tet(M) and erm(B) respectively. But the primers used are different including the cyclic parameters (Gad, Abdel-Hamid and Farag, 2014).

Table 6. tet(M) (tetracycline) and erm(B) (erythromycin) specific primer sequences and product size

Primer Set Sequence (5' to 3')Product size Reference

Tet (M) forward GGTGAACATCATAGACACGC

401

(Gad, Abdel-Hamid and Farag, 2014).

Tet (M) reverse CTTGTTCGAGTTCCAATGC

Erm (B) forward CATTTAACGACGAAACTGGC

405

Erm (B) reverse GGAACATCTGTGGTATGGCG

Table 7. Amount of DNA and Water need for each sample for the PCR reaction for Kit Based extraction method.

| Methods | Sample | Amount of PCR Mater mix added(µl) | Amount of DNA added(µl) | Amount of water added(µl) |
|---|---|---|---|---|
| Boil cell method | Negative control | 12.25 | - | 12.75 |
| | Sample 2 | 12.25 | 2.0 | 10.75 |
| | Sample 4 | 12.25 | 0.3 | 12.45 |
| Kit Based extraction | Negative control | 12.25 | - | 12.75 |
| | Sample 2 | 12.25 | 2.1 | 10.65 |
| | Sample 4 | 12.25 | 8.5 | 4.25 |

The separate cyclic parameters published by Gad, Abdel-Hamid and Farag, 2014 are given below in Table 8 and Table 9.

*Table 8. PCR cyclic parameters for erm(B)*

| Step | Temperature (°C) | Time/min | Cycles |
|---|---|---|---|
| Initial denaturation | 94 | 5 | |
| Denaturation | 94 | 1 | 35 |
| Annealing | 52 | 1 | |
| Extension | 72 | 2 | |
| Final Extension | 72 | 12 | |
| Final hold | 4 | | |

*Table 9. PCR cyclic parameters for tet(M)*

| Step | Temperature (°C) | Time/min | Cycle times |
|---|---|---|---|
| Initial denaturation | 94 | 5 | |
| Denaturation | 94 | 1 | 35 |
| Annealing | 55 | 1 | |
| Extension | 72 | 2 | |
| Final Extension | 72 | 12 | |
| Final hold | 4 | | |

**Detection by agarose gel electrophoresis**

With aseptic conditions, 2% agarose gel was prepared and 8µl of each samples and negative control was loaded onto the wells followed by 2µl of the DNA ladder (100bp). Then the gel was run at 45V for 15 minutes and 55V for 45 minutes. This protocol was followed for all PCR related methods in this stud

## *RESULTS*

Morphological identification of Streptococcus growth on MRS media

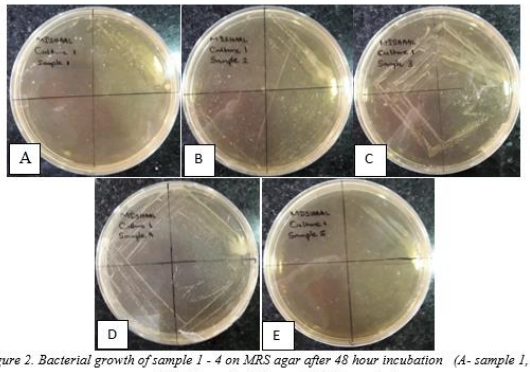


*Figure 2. Bacterial growth of sample 1 - 4 on MRS agar after 48 hour incubation (A- sample 1, B- sample 2, C-sample 3, D-sample 4, E-sample 5)*

As shown in Figure 2 Creamy colour, circular, relatively small colonies were observed in MRS agar. Less growth were observed in A and B and in C and D the growth was higher. There were no colonies observed in sample 5 and there were no contaminations present in any agar media.

Gram staining results of Streptococcus culture

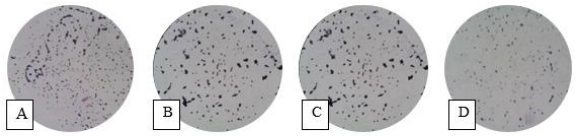


*Figure 3. Bacterial identification by Gram staining images of sample 1 - 4 under 100X magnification (A- sample 1, B-sample 2, C-sample 3, D-sample 4)*

The isolated bacteria shown in Figure 3 were purple in colour (Gram positive) cocci bacteria, there were cocci chains, diplo cocci and mostly single cocci in the smear

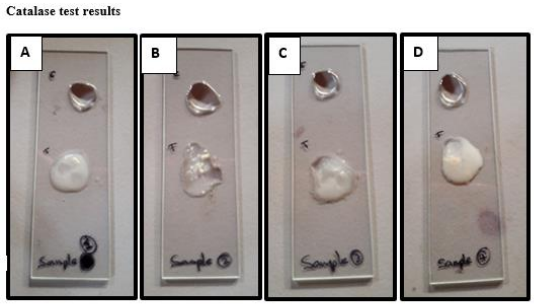


*Figure 4. Catalase test results of all the sample A- sample 1, B-sample 2, C-sample 3, and D-sample 4. All the samples showed negative results for catalase test.*

According to Figure 4. there were no bubbles present in all the samples in the presence of H2O2 and it confirms all the samples contain anaerobes. Furthermore, it confirms there is no Staphylococcus present in the samples.

Morphological identification of Streptococcus Sub culturing pure colonies in MRS agar

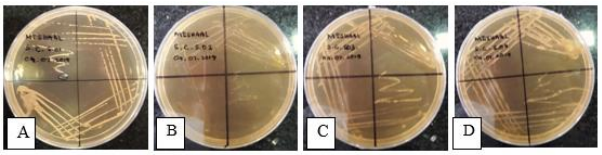


*Figure 5. Streptococcus sub culture growth of sample 1-4 on MRS agar*

As shown in ***Figure 5*** Creamy colour, relatively small and circular colonies in high numbers of growth were observed in MRS media.

Subculture growth under different temperatures

Table 10. Subculture growth under 37ºC and 45ºC for 24 hour incubation time

*Table 10. Subculture growth under 37ºC and 45ºC for 24 hour incubation time*

| Sample | Growth at 37°C | Growth at 45°C |
|---|---|---|
| S1 | ✓✓ | ✓ |
| S2 | ✓✓ | ✓✓ |
| S3 | ✓✓✓ | ✓ |
| S4 | ✓✓✓ | (-) |

No growth - (-), Less growth - ✓, Normal growth - ✓✓, High growth -✓✓✓

With the results mentioned in Table 10 at 37oC growth was observed in 4 samples and only 3 samples were grown in 45oC incubation specifically sample 4 didn't have any growth but streptococcus optimum temperature is between 37oC – 45oC. Sample 3 and sample 4 has maximum growth at 37oC.

Spectrophotometer results for DNA quantification

Table 11. Mean absorbance values for boiled cell method.

| Sample | Mean Absorbance at 230nm | Mean Absorbance at 260nm | Mean Absorbance at 280nm |
|---|---|---|---|
| Sample 1 | 0.0173±0.0006 | 0.0287±0.0006 | 0.0137±0.0006 |
| Sample 2 | 0.0070±0.0010 | 0.0100±0.0000 | 0.0053±0.0006 |
| Sample 3 | 0.0403±0.0006 | 0.0767±0.0006 | 0.0420±0.0000 |
| Sample 4 | 0.0437±0.0006 | 0.0763±0.0006 | 0.0390±0.0000 |

*Table 12. Mean absorbance values for kit based extractions.*

| Sample | Mean Absorbance at 230nm | Mean Absorbance at 260nm | Mean Absorbance at 280nm |
|---|---|---|---|
| Sample 1 | 0.0047±0.0006 | 0.0080±0.0000 | 0.0043±0.0006 |
| Sample 2 | 0.0053±0.0006 | 0.0093±0.0006 | 0.0050±0.0000 |
| Sample 3 | 0.0030±0.0000 | 0.0047±0.0012 | 0.0020±0.0010 |
| Sample 4 | 0.0013±0.0006 | 0.0023±0.0006 | 0.0010±0.0000 |

According to Table 11 and Table 12 Mean absorbance at 230nm, 260nm, 280nm sample 1-4 in Boil cell method is higher and Kit based method shows less values compared to boil cell method. Highest values are from sample1, 3 and 4 in boil cell method.

Table 13. Mean concentration values for both boil cell and kit based extractions methods.

| Sample | Mean DNA Concentrations At 260nm ($ngul^{-1}$) Boil cell method | Mean DNA Concentrations At 260nm ($ngul^{-1}$) Kit based extractions |
|---|---|---|
| Sample 1 | 144.77±2.915619 | 40.40±0 |
| Sample 2 | 50.50±0 | 47.13±2.915619 |
| Sample 3 | 387.17±2.915619 | 23.57±5.831238 |
| Sample 4 | 385.48±2.915619 | 11.78±2.915619 |

According to Table 13 Mean concentration values are higher in sample 1, 3 and 4 boil cell method and in kit based method comparatively sample 2 has the higher value. When comparing both methods all the DNA Concentrations values are higher in boil cell method which are higher than kit based method.

Table 14. Table depicting the DNA purity ratios for Boiled cell and kit based extraction methods.

| Boil cell extraction method | | | Kit based extraction method | | |
|---|---|---|---|---|---|
| Sample | 260/230 Mean Absorbance ratio | 260/280 Mean Absorbance ratio | Sample | 260/230 Mean Absorbance ratio | 260/280 Mean Absorbance ratio |
| Sample 1 | 1.65±0.048 | 2.10±0.118 | Sample 1 | 1.71±0.231 | 1.85±0.231 |
| Sample 2 | 1.43±0.209 | 1.88±0.192 | Sample 2 | 1.75±0.252 | 1.87±0.115 |
| Sample 3 | 1.90±0.023 | 1.83±0.014 | Sample 3 | 1.56±0.385 | 2.33±1.155 |
| Sample 4 | 1.75±0.020 | 1.96±0.015 | Sample 4 | 1.75±1.000 | 2.33±0.577 |
| Standard values | 2.0-2.2 | 1.8 – 2.0 | Standard values | 2.0-2.2 | 1.8 |

If the 260/280 ratio value is ≥ 1.8 it is consider as pure DNA if its < 1.8 it can be concluded that there is a protein present in the DNA sample, if the ratio is ≥ 2.0 it confirms the presence of RNA in the sample. If the 260/230 ratio is ≥ 2.0-2.2 its considered as pure if its less the value its due to the presence of a organic solvent in the sample. With regard to these information and according to Table 14 all the 260/280 ratio value in boil cell and kit based extraction are above the standard therefore it is considered as pure. But in

boil cell sample 1 and kit based extractions sample 3 and 4 ratio values are > 2.0, this confirms the presence of RNA in the sample. All the 260/230 ratio values are lower than the standard value therefore it confirms the presence of organic solvents.

Statistical analysis using one-way **ANOVA**

Table 15. Comparison of DNA yield between methods and brand( sample)

**Tests of Between-Subjects Effects**

Dependent Variable: DNA yield

| Source | Type III Sum of Squares | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Corrected Model | 476227628.156[a] | 7 | 68032518.308 | 6467.104 | .000 |
| Intercept | 407793582.094 | 1 | 407793582.094 | 38764.455 | .000 |
| Method | 236225513.344 | 1 | 236225513.344 | 22455.364 | .000 |
| Brand | 94481980.781 | 3 | 31493993.594 | 2993.788 | .000 |
| Method * Brand | 145520134.031 | 3 | 48506711.344 | 4611.000 | .000 |
| Error | 168316.500 | 16 | 10519.781 | | |
| Total | 884189526.750 | 24 | | | |
| Corrected Total | 476395944.656 | 23 | | | |

a. R Squared = 1.000 (Adjusted R Squared = .999)

With respect to the Table 15 when comparing DNA yield between method and brand (sample) individually and both method and brand (sample) together all has a p – value less than 0.05(0.000 for all) respectively indicating there is a statistically significant difference between the DNA yields.

**Multiple Comparisons**

Dependent Variable: DNA yield

Tukey HSD

| (I) Brand | (J) Brand | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound |
| S1 | S2 | 1186.7500* | 59.21650 | .000 | 1017.3304 | 1356.1696 |
| | S3 | -3383.5000* | 59.21650 | .000 | -3552.9196 | -3214.0804 |
| | S4 | -3181.5000* | 59.21650 | .000 | -3350.9196 | -3012.0804 |
| S2 | S1 | -1186.7500* | 59.21650 | .000 | -1356.1696 | -1017.3304 |
| | S3 | -4570.2500* | 59.21650 | .000 | -4739.6696 | -4400.8304 |
| | S4 | -4368.2500* | 59.21650 | .000 | -4537.6696 | -4198.8304 |
| S3 | S1 | 3383.5000* | 59.21650 | .000 | 3214.0804 | 3552.9196 |
| | S2 | 4570.2500* | 59.21650 | .000 | 4400.8304 | 4739.6696 |
| | S4 | 202.0000* | 59.21650 | .017 | 32.5804 | 371.4196 |
| S4 | S1 | 3181.5000* | 59.21650 | .000 | 3012.0804 | 3350.9196 |
| | S2 | 4368.2500* | 59.21650 | .000 | 4198.8304 | 4537.6696 |
| | S3 | -202.0000* | 59.21650 | .017 | -371.4196 | -32.5804 |

Based on observed means.

The error term is Mean Square(Error) = 10519.781.

*. The mean difference is significant at the 0.05 level.

According to Table 16 when comparing DNA yield between brands (samples) together all has a p – value less than 0.05 respectively indicating there is a significant difference between the DNA yields obtained from different samples used.

**Comparison of DNA yields**

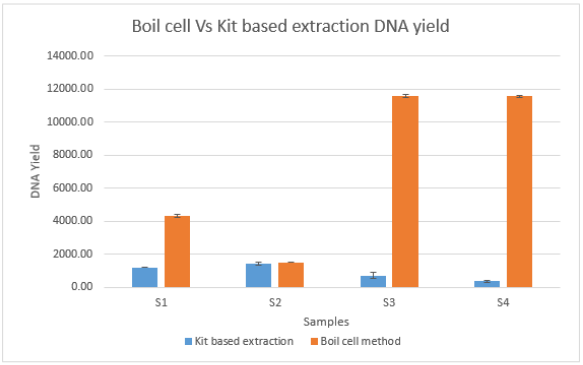


*Figure 6. Comparison between two extraction methods using DNA yield*

As shown in Figure 6 Boil cell methods show high DNA yield in sample 3 & 4. Generally, boil cell method gave highest DNA yield compared to kit based method.

Identification of Streptococcus with polymerase chain reaction using DNA extracts from Boil cell method and kit based extraction methods

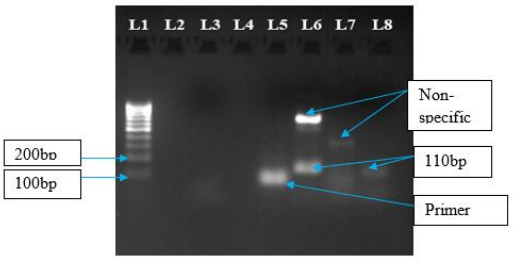


*Figure 7. Amplified PCR products of Sample 1-4 of boil cell method to identify Streptococcus in 2% agarose gel. In Lane 1-100bp ladder, Lane 3- negative control, Lane 5- sample 1, Lane 6- sample 2, Lane 7- sample 3 and Lane 8- shows sample 4.*

According to Figure 7 110bp pair length Products were observed in sample 2 and 4. Therefore, sample 2 and 4 contains Streptococcus. Negative control was not contaminated. Non-specific bands were observed on sample 3 and 4, primer dimers were observed on sample 1.

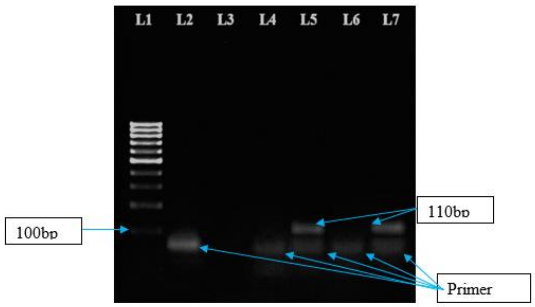


*Figure 8. Amplified PCR products of Sample 1-4 of kit based extraction method to identify Streptococcus in 2% agarose gel. In Lane 1-100bp ladder, Lane 2- negative control, Lane 4- sample 1, Lane 5- sample 2, Lane 6- sample 3 and Lane 7- shows sample 4.*

According to Figure 8 110bp pair length Products were observed in sample 2 and 4. Therefore, sample 2 and 4 contains Streptococcus. Negative control was not contaminated. Only primer dimers were also observed on all the samples.

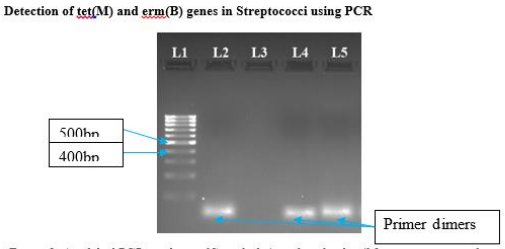


*Figure 9. Amplified PCR products of Sample 1-4 to identify of tet(M) gene using specific primer in 2% agarose gel. In Lane 1-100bp ladder, Lane 2- negative control, Lane 4- sample 2 and Lane 5- shows*

sample 4

According to Figure 9. 401bp pair length Products were not observed in sample 2 and 4. Therefore, sample 2 and 4 does not contains tet(M). Negative control was not contaminated. Only primer dimers were observed in both the samples.

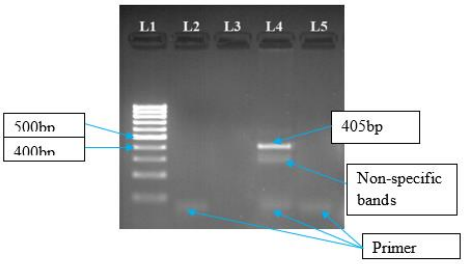


*Figure 10. Amplified PCR products of Sample 1-4 to identify of erm(B) gene using specific primer in 2% agarose gel. In Lane 1-100bp ladder, Lane 2- negative control, Lane 4- sample 2 and Lane 5- shows sample 4*

According to Figure 10 405bp pair length Products were observed in sample 2. Therefore, sample 2 contains erm(B) and sample 4 did not have a band and its negative for erm(B). Negative control was not contaminated. A non-specific band was observed in sample 2 and primer dimers were observed.

## *DISCUSSION*

Lactobacillus spp. and Bifidobacterium spp. are the two main types of probiotis, isolated mainly from dairy products. The present study was focused to identify Streptococcus spp. by its probiotic characteristics and to observe its antibiotic resistance. Table olives are a natural source of probiotic bacteria and are well known for their health promoting useful products (Bonatsou et al., 2017). In the previous studies, streptococcus was grown in M17 agar (Yang et al., 2018; Guevarra and Barraquio, 2015; Collado and Hernández 2006; Mater et al., 2005), but in this study De Man Rogosa and Sharpe (MRS Agar) was used instead and colony growth was observed. The purpose of using MRS agar was to identify the presence of other probiotic bacteria in fermented olive and to find Streptococcus to subculture, and 4 out of 5 samples had a growth. Sample 5 did not show a growth, suggesting there is no lactic acid bacteria

present in the sample. Bacterial growth of the samples were creamy and opaque in colour, the colonies were relatively small and circular in appearance as shown in (Figure 2). The colonies obtained had the same characteristic for Streptococcus colony appearances in study conducted by Guevarra and Barraquio in 2015; hence further tests were carried out in 3 different stages to confirm the presence of Streptococcus. Gram staining followed by the catalase test and growth under different temperatures was tested to identify Streptococcus spp. in the samples. Since sample 5did not have any colony growth, further tests were not performed on the particular sample. Gram staining of all the samples (Figure 3) showed gram positive coccus only and mainly chains, pairs and single cocci and fewer clusters were also observed, in dark purple colour.

The intention of the Catalase test is to find the action of catalase enzyme that transforms hydrogen peroxide into H2O and oxygen. Usually aerobic or facultative anaerobic bacteria contain this enzyme, thus the Catalase test was carried out with the intention of finding the presence of catalase enzyme in bacteria (Iwase et al., 2013). The samples were found to be catalase negative and it confirms that all the bacteria present are anaerobes as shown in Figure 4 and there is no Staphylococcus growth in the samples. With this relevant information sub culturing was carried out in MRS agar plates and the agar plates had good yield of bacteria. Pure colonies were sub cultured in MRS agar and incubated under two temperatures respectively 37oC and 45oC. As shown in Table 10, only 3 samples had growth in 45oC which had only cream colour colonies while the sample 4 did not grow under 45°C, and all the 4 samples had growth under 37oC where the sample 1 had creamy and opaque colour colonies while the rest were all cream colour. Similar results were shown in a study carried out by Gad,Humaid and Farag (2014).Streptococcus will grow in 37oC and 45oC but the study by Radke-Mitchell and Sandine, (1986) have shown the growth between 30oC and 42oC and maximum growth at 43oC-46oC. Lactococcus have growth in 15oC and 37oC with related to this sample 4 can be Streptococcus or no growth can be due to a growth inhibition.

For further confirmations, Polymerase Chain Reaction (PCR) was used. DNA extraction was carried out in 2 different methods to obtain DNA for the process, namely boil cell extraction and Promega kit based extraction method out using the study Abdulamir et al., (2010).PCR requires a specific amount of DNA concentration for ideal performance. This helps to prevent unnecessary consumption, enhance reproducibility and amplification of the samples (Boesenberg-Smith, Pessarakli and Wolk, 2012). A study was done to find the accuracy of DNA concentration with DNA samples using spectrophotometric DNA quantification, and compared it with fluorometric quantification method. Spectrophotometric DNA quantification was the most precise method (Haque et al., 2003) and this was supported by a study carried out by Shokere, Holden and Jenkinsin (2008).

Absorbance at 230nm, 260nm and 280nm were obtained in triplet values using spectrophotometer and mean was calculated. All the samples, especially sample 3 and 4 had high values in Boil cell extraction absorbance values confirming the presence of high DNA yield. After thatA260/A230 ratio and A260/A280 ratio was calculated with the DNA absorbance mean and compared with the standard values of the ratios A260/A280 standard was 1.8 -2.0 (Desjardins and Conklin, 2010) or above values if it's less than that it confirms the presence of protein but all the values from both extraction methods had values passing standard values

showing higher values and but values obtained by boil cell method had purity. Sample 1 in boil cell method and sample 3 and 4 in kit based method had above A260/A280 ratio above 2.0 and it confirms the presence of RNA. A260/A230 ratio standard is above 2.0 -2.2 (Aphale and Kulkarni, 2018) whereas no sample had that value, thus confirms the presence of organic solvents. According to the graph shown in (Figure 6) boil cell method has a higher DNA yield compared to Promega kit based extraction method.

the study carried out by Abdulamir et al., in (2010) shows higher purity and DNA yield was obtained from Promege kit method compared to boil cell method but in another study done by Peng et al., (2013) confirms boil cells method has similar DNA yield like other kits methods.

According to SPSS data suing one way ANOVA results in the Table 15 comparing DNA yield between method and brand (sample) and both method and brand (sample) together all has a p – value less than 0.05(0.000 for all) respectively indicating there is a significant difference between the DNA yields of brand and methods because the yield obtained from boil cell method is higher than Promege kit based method. With relevant to the data in Table 16 DNA yield between brands (samples) together all has a p – value less than 0.05 respectively indicating there is a significant difference between the DNA yields and samples used since the bacteria concentration available in each sample is different from one other.

In order to further confirm the presence of Streptococcus spp. and to identify, molecular based techniques were used along with extracted quantified DNA and genus specific primers for streptococcus. According to the study carried out by Prabhu et al., (2012) expected amplicon size is 110bp.Nevertheless, with the lower values of A260/A230 ratios and proper A260/A280ratios positive bands were observed between 100bp and 200bp in sample 2 and 4 in both boil cell and Promega kit based extraction methods. Nonspecific binding was observed in boil cell method and not in kit based method. The annealing temperature of the primers should be optimized to prevent the formation of primer dimers. In addition there were no bands observed in negative control and can be concluded there is no contaminations (Ali et al., 2014). A study was carried out by Preethirani et al., (2015) using same primer sequence (tuf) to identify Streptococcus and out of 5 samples all gave positives and there were no primer dimers present, the only difference was annealing temperature they used which was 50oC.

PCR was performed to detect the presence of tet(M) and erm(B) in sample 2 and 4.Molecular methods are more sensitive and reliable compared to other conventional methods and it is less time consuming as well, and that is why molecular methods were utilized in the present study to confirm the results (Wong et al., 2015). As shown in (Figure 9) no band was observed at 400bp level and it confirms all the samples are negative for tet(M) gene and since negative control did not show a band it can be concluded that the reagents and samples are not contaminated. Since there was a band which is 400bp in sample 2 (Figure 10) that is the only sample which is positive for erm(B) while sample 4 was negative for erm(B) gene and the negative control wasn't contaminated. With these results it can be concluded that Streptococcus can have antibiotic resistance for Erythromycin (Zheng et al., 2017).

This study demonstrated that presence of Streptococcus as a probiotic in olive samples and its antibiotic resistance. Out of all the 5 samples, sample 2 and 4 were streptococcus positive and sample 2 was resistant for erythromycin and susceptible for tetracycline. Antibiotic resistance has become urgent threat to public health and despite the health benefits some probiotics

have reported to harbour resistance and these can be transferred to other bacteria. In order to prevent these it should be safely engineered to commercialize and to be used for next generation.

## *CONCLUSION*

This study aimed to isolate Streptococcus from table olive samples. The boil-cell method was proved to be a better extraction procedure since it produced a greater DNA yield compared to kit-based method. PCR amplification indicated the presence of Streptococci bacteria, further confirming the gram staining results. The detection of tet(M) and erm(B) genes using PCR showed positive results for the sample 2, confirming the presence of erm(B) gene. However, none of the samples possessed the tet(M) gene. Further analysis of other resistant genes are required as there is a possibility of resistant genes to transfer and pose a threat to the human health.

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