A COMPARATIVE STUDY OF THE RELATION BETWEEN BOD AND MICROBIAL COUNT IN DIFFERENT WELL WATER SAMPLES.

Project Report Submitted to Sacred Heart College (Autonomous), Affiliated to Mahatma Gandhi University, Kottayam, in Partial Fulfillment of the Requirements for the

Degree of Bachelor of Science in Zoology

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STUDY ON WATER QUALITY PARAMETERS OF PAMPA RIVER (LOWER REACHES) AND ADJACENT WELLS

Dissertion submitted to

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THE EFFECT OF FEED ON THE GROWTH RATE OF TILAPIA (*Oreochromis niloticus*)

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STUDY OF ANTIMITOTIC EFFECT OF *CURCUMA LONGA* AND *AZADIRACHTA INDICA*

Dissertation submitted to the

Sacred Heart College (Autonomous) Thevara

In Partial fulfillment of the Degree of Bachelor of Science in Zoology

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2016-2019

CERTIFICATE

This is to certify that this project work entitled "STUDY OF ANTIMITOTIC EFFECT OF *CURCUMA LONGA* AND *AZADIRACHTA INDICA*, is an authentic record of research work carried out by Ms. NEILAH FIRZAN C A, Reg No: 16UZOO4860, of this department as a part of BSc (Zoology) degree programme during the year 2016-2019.

Place: Thevara

Date:

Head of the Department

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CANDIDATE'S STATEMENT

I hereby declare that the work incorporated in the present dissertation is original and has not been submitted to any institution for the award of any diploma or degree.

I further declare that the results presented in the dissertation, consideration made therein, contribute in general to the advancement of knowledge of science.

Place: Thevara Signature of Candidate

Date:

CERTIFICATE BY THE GUIDE

This is to certify that the contents of this dissertation entitled "STUDY OF ANTIMITOTIC EFFECT OF *CURCUMA LONGA* AND *AZADIRACHTA INDICA*" is the original research work of Ms. NEILAH FIRZAN C A, carried out under my supervision.

I further certify that the work has not been submitted either fully or partially to any other University or Institution for any degree.

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CONTENTS

ABSTRACT

Medicinal herbs have been used in folk medicine for millennia. Simply in recent times, scientific study of their effects have flourished. Never the less, some of them can cause adverse effects or have the potential to interact with other medications. Moreover, there is little information on the potential risk to health of such herbs. Based on their long term use by human one might expect herbs used in traditional medicine to have low toxicity. So the aim of my project is to observe the effects of *Azadirachta indica* and *Curcuma longa* on the root tip of *Allium cepa* (onion) and to prove that these extracts have potential anti-cancer property.

In order to prove that, mitotic index of the onion root treated with extracts of *Azadirachta indica* and *Curcuma longa* were observed and compared with the mitotic index of untreated onion root tip which was considered as the control.

Results obtained showed that the plant extracts have an effect on the mitotic index. As the concentration of the extract increased, the mitotic index decreased, in comparison with the control. This may be due to antiproliferating property of the extracts. Thus this could be very beneficial in the treatment of cancer as plant extracts could possibly stop cancerous cells from dividing and can be used as natural source of anti-cancer drug.

INTRODUCTION

The general principles of the mechanism of mitosis are best and most easily studied in the actively growing regions of plants such as shoot or root apex. A wide variety of secondary metabolites obtained from plants are tested for their ability to treat cancer. Various anticancer drugs from plants are known to be effective against proliferating cells. They exhibit cytotoxic effect by interfering with cell cycle kinetics. These drugs are effective against cells that are proliferating and produce cytotoxic effect either by damaging the DNA during the S-phase of the cell cycle or by blocking the formation of the mitotic spindle in M-phase. However most of the cytotoxic drugs exhibit side effects, and hence, there is a need for drugs that are efficient and have less side effects

In *Allium cepa L.* root tip model root system of plant cells is commonly used as a test for investigating environmental pollution factors, toxicity of chemical compounds and evaluating potential anticancer properties. It is very comfortable as it is easy to make preparations of onion roots. They can contain rather homozygous meristematic cells, having only 16 chromosomes, which are very long, well visible and get stained easily. The test is fast and inexpensive method.

Mitotic index and significance

Mitotic index is defined as the ratio between the numbers of cells in a population undergoing mitosis to the number of cells in a population not undergoing mitosis. The purpose of the mitotic index is to measure cellular proliferation. The M.I is an important prognostic factor predicting both overall survival and response to

chemotherapy in most types of cancer. Cancer cells have higher M.I because they have a mutation in the DNA so they reproduce uncontrollably.

M.I can be calculated as:

M.I = (number of cells in mitosis/total number of cells) × 100

The cell cycles or cell division is the series of events that takes place in a cell leading to its division and duplication of its DNA to produce two daughter cells. The four major phases of this process include G1 phase, S phase, G2 phase and M phase, where mitosis occurs. All cell must replicates their DNA when dividing. During DNA replication, the two strands of the DNA double helix separate and for each original strand a new complementary strand is produced. DNA replication in eukaryotes is followed by a process called “mitosis”, which assures that each daughter cell receives one copy of each of the replicated chromosomes. Mitosis can be broken down into several phases including prophase, metaphase, anaphase and telophase. The actual division of cytoplasm is called cytokinesis and occur during telophase.

THE STAGES OF MITOSIS

Prophase: in prophase the centrosomes begin to move to opposite poles of a cell. The microtubules that form the basis of the mitotic spindle extend between centrosome, pushing them farther apart as the microtubule fibers lengthen. The sister chromatids begin to coil more tightly and become visible under light microscope. At the centromere region of each chromosome, a large protein

complex called kinetochore serves as a site for attachment to microtubules. And in late prophase, lamins and inner nuclear membrane are phosphorylated, causing the nuclear lamina and nuclear pore complexes to disassemble and disperse in cytoplasmic membrane vesicles.

Metaphase: Metaphase represents the stage at which chromosomes are aligned in a plane forming the equatorial plate or metaphase plate. The sister chromatids are held together only at their common centromere. Associated with the centromere is a proteinacious structure called the kinetochore. It is to the kinetochore that the chromosomal microtubules of the spindle are attached.

Anaphase: Anaphase begins with the splitting apart of centromere of the two sister chromatids. Two sister chromatids become separate structures and can be called daughter chromosomes. This migrates to the opposite poles. The kinetochore leads the rest of the chromatids being pulled by the spindle fibers attached to it.This pulling causes the chromosomes to assure the characteristic V or L –shape. During anaphase the microtubules attached to the kinetochore shorten from one third to one fifth of the original length. At the same time the microtubules situated in between the poles elongate.

Telophase: The end of polar migration of the daughter chromosomes marks the beginning of telophase. Telophase is characterized by the uncoiling and dispersal of the chromatin of each chromosomes and by the reformation of the nucleolus and nuclear envelope, derived from endoplasmic reticulum. Astral and spindle fibers disappears. Simultaneously with these process cytokinesis occurs.

Cytokinesis: It is the process of segmentation of cytoplasm. It is different in plant and animal cells. This process divides uncontrollably causing cancer.

Certain alkaloid drugs and natural products were studied with the hope of inhibiting the uncontrolled division of cells. Medicinal plants also have certain alkaloids which are capable of inhibiting uncontrolled division of cells. Alkaloid drugs have the ability to inhibit microtubule polymerization by binding to the protein tubulin, which leads to microtubule degradation. This disrupts mitosis and leads to cell death because microtubules make up the mitotic spindles, which allow eukaryotic cells to separate their chromatids during cell division. Without microtubules, the cell will not divide. The mitotic index becomes lower. This could be very beneficial in the treatment of cancer.

In the present study, extracts of two plants having anticancer property were used, leaf extract of *Azadirachta indica* and root extract of *Curcuma longa.*

Azadirachta indica:

SCIENTIFIC CLASSIFICATION:

Kingdom	Plantae
Order	Sapindales
Family	Meliaceae
Genus	*Azadirachta* A. Juss.
Species	*Azadirachta indica* A. Juss.

Azadirachta indica commonly called as neem is one of the most versatile medicinal plant. It is a rich source of limonoids that are endowed with potent

medicinal properties predominantly antioxidant, anti-inflammatory and anti-cancer activities. Azadirachtin, gedunin and nimbolide are more extensively investigated relative to other neem limonoids. Anticancer effects of neem limonoids are mediated through the inhibition of cell proliferation, apoptosis evasion, inflammation and angiogenesis.

Curcuma longa:

SCIENTIFIC CLASSIFICATION:

Kingdom	Plantae
Order	Zingiberales
Family	Zingiberaceae
Genus	*Curcuma* L.
Species	*Curcuma longa* L.

Curcuma longa commonly called as turmeric is a widely used popular Indian medicinal plant. Curcumin is an active constituent of turmeric which is polyphenolic compound. Curcumin is known for its four main medicinal property- anti-inflammatory, antioxidant, antibacterial and anticancer. It regulates not only the various pathways of the immune system, cell cycle checkpoints, apoptosis and antioxidant response but also numerous intracellular targets, including pathways and protein molecules controlling tumor progression. Curcumin has been suggested to inhibit cell proliferation by diverse mechanism. Curcumin bind to purified tubulin and inhibit tubulin polymerization and also found to perturb the microtubule spindle structure. Curcumin is also found to affect the activity of the chromosomal passenger complex, resulting in multipolar

chromosome segregation promoting mitotic catastrophe. Curcumin inhibits cell proliferation by inhibiting microtubule dynamics.

AIM

To determine the effect of extract of neem leaf and extract of turmeric root on mitosis using *Allium cepa* root cells.

OBJECTIVES

- To find the mitotic index of untreated *Allium cepa* root tip cells.
- To find the mitotic index of *Allium cepa* root tip cells after treatment with extract of *Azadirachta indica*.
- To find the mitotic index of *Allium cepa* root tip cells after treatment with extract of *Curcuma longa*

REVIEW OF LITERATURE

Natural products are a source of therapeutic drugs and are used by physicians of indigenous systems of medicine for over hundreds of years. The standard herbal preparations mostly consist of complex mixtures of one or more plants which are used in most countries (Calixio, 2000). In order to initiate the search for drugs from plants, the antimitotic activity of the extracts were tested by *Allium cepa* assay (Levan,1949). The *Allium cepa* root meristem assay is considered widely as a practical and reliable system for the screening of environmental mutagens and carcinogens (Fiskc - jo, 1985; Stich *et al*., 1975). As the patterns of divisions in onion cells and animal somatic cells are similar, an extract which is able to inhibit the cell division in *Allium cepa* root cells, will be effective in human and animal cells. Thus it is possible that chemicals that affect plant chromosomes will also affect the chromosomes of animals. Hence these meristematic cells of plants can be used for preliminary screening of antimitotic / anticancer activity of extracts / drugs (Williams, 1996). The onion root tip assay is used by many researchers to screen several plant extracts to evaluate their antimitotic activity.

Cytotoxic assays were conducted on a wide spectrum of plants by several earlier workers using different test materials. Shehab (1979) studied the cytological effect of water extract of Pulicaria nispa on *Allium cepa*. The extract affected the mitotic index and percentage of the mitotic stages in treated roots. The percentage of anomalies increases with increase of concentration and duration of treatment.

The abnormalities were spindle disturbances, stickiness, bridges and laggards. In 1980, Shehab reported the antimitotic effect of water extract from *Teucrium pilosum* on *Allium cepa*. The induced cytotoxic effects include stickiness, C-mitosis, laggards, bridges, polyploidy and chromosomal breaks.

Mitotic effects of aqueous leaf extract of *Cymbopogon citratus* were demonstrated on *Allium cepa* root tips (Williams, 1996). A steroidal drug, sarsapogenin was tested for its cytotoxicity and antimitotic activity on root tip meristematic cells of *Allium cepa*. The drug was found to possess profound effect on mitotic spindle inhibition and chromosomal abnormalities during prolonged treatment (Sinha, 1996).

Veronica *et al*. (2001) reported the effect of medicinal tea prepared from *Averrhoa carambola*, *Syzygium cuminum* and *Cissus sicyoides*. The results showed that tea did not alter the cell cycle of *Allium cepa*. But lower concentrations after 24 hr. treatment showed decrease in the mitotic index.

The genotoxic effect of an aqueous extract of neem was evaluated using *Allium cepa* chromosome aberration assay. Neem extracts suppressed the mitotic activity of Allium root meristems after 24-48 hr. treatment with all concentrations. The extracts caused different kinds of chromosome aberrations in dividing and non-dividing cells of *AIIium cepa* such as micronucleus, multinucleated cells in the interphase stage, bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed anaphase (Soliman, 2001).

Cytotoxicity of plant extracts on in vitro cell lines cytotoxic components of *Zingiber zemmbet*, *Curcurna zeodaria* and *C. domestica* were studied by Matthes *et al.* (1980). Root extracts of the three species of Zingiberaceae showed marked

cytotoxicity against neoplastic cells. One new compound (3",4"-o-diacetyl afzelin) and five known compounds (zerumbone, zerumbone epoxide and the curcuminoids, diferuloyl methane, feruloyl-p-coumaryl methane and di-p-coumaryl-methane) were isolated and all these were found to be cytotoxic.

The cytotoxic effect of eight synthetic curcuminoids on L929 cells were reported by John *et al*. (1996). All synthetic curcuminoids used in the study were found to be cytotoxic against cancer cells.

Ethanolic extracts of forty three Jordanian medicinal plants were examined for cytotoxicity. Among them, *Curcuma longa* and *Zingiber officinale* showed cytotoxicity against A549 cancer cells, MCF-7 female breast carcinoma and HT 29 colon adenocarcinoma cell lines (Alkofahi, 1997).

Cytotoxicity of curcuminoids and some novel compounds from *Curcuma zeodaria* were reported earlier by Syu *et al*. (1998). Extracts of roots of *C. zeodaria* led to the isolation of the curcuminoid, identified as dimethoxy curcumin. Cytotoxicity was exhibited against human ovarian cancer cells.

Methanolic extracts, water extracts and volatile oils of fresh rhizomes of *Alpinia galanga, Bosenbergia pandurata, Curcuma longa* and *Zingiber officinale* have been assessed for cytotoxic activity against MCF-7 breast adeno-carcinoma and LS 174T - colon adeno-carcinoma cell lines by Zaeoung *et al*. (2005). The results showed that methanolic extract of Curcuma longa showed strong activity against MCF 7 and LS 174 T cell lines, whereas the water extracts of these plants exhibited slight cytotoxic activity. All volatile oils and methanol extracts were capable of inhibiting proliferation of the two cell lines. It is notable that volatile

oils of these rhizomes were mainly composed of monoterpenes, sesquiterpenes and phenyl propanoids and could be responsible for the cytotoxic activity.

Kalpana *et al.* (2004) reported the modulating effects of curcumin on lipid peroxidation and antioxidant status during nicotine induced toxicity. The results indicated that administration of curcumin reversed the changes induced by nicotine, supporting the hypothesis that plant products are effective antioxidant agents.

Essential oils are-valuable natural products used as raw materials in perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutraceuticals (Buchbauer, 2000). The volatile oils are complex mixtures comprising several compounds. Each of these constituents contribute to the beneficial or potential effects of these oils. Thus the intimate knowledge of essential oil composition allows for a better and specially directed application (Buchbauer, 2000). Considering all the above mentioned difference in essential oil composition it is clear that only a detailed knowledge of the constituents of the essential oil will lead to a proper use in cosmetics by perfumers and cosmetic chemists. Such a detailed knowledge can be obtained.

The genotoxic effect of an aqueous extract of neem was evaluated using *Allium cepa* chromosome aberration assay. Neem extracts suppressed the mitotic activity of Allium root meristems after 24-48 hr. treatment with all concentrations. The extracts caused different kinds of chromosome aberrations in dividing and non-dividing cells of *AIIium cepa* such as micronucleus, multinucleated cells in the interphase stage, bridges, stickiness, non-congression metaphase, laggards, polyploidy and disturbed anaphase (Soliman, 2001).

METHODOLOGY

MATERIALS REQUIRED:

- Onion root tip
- Toluidine blue stain
- 6N HCl
- Leaf extract of *Azadirachta indica* and root extract of *Curcuma longa*.
- Petri plates
- Needle and brush
- Water and blotting paper
- Microscopic slides and coverslip
- Light compound microscope
- Glycerin
- Alcohol
- Test tubes
- Test tube holder
- Dropper

PROCEDURE

Four bulbs per concentration were used. The bulbs were placed on a glass test tube containing distilled water and allowed to germinate at room temperature. As soon as the roots were about 2-4 cm long they were treated with different concentrations of *Curcuma longa* and *Azadirachta indica* for 24 hrs.

Preparation of extract: Fresh leaves of *Azadirachta indica* were collected, rinsed and air dried at room temperature by spreading them for 24 hrs. 100g of fresh leaves were weighed, blended and soaked in 50 ml of distilled water for 24 hrs. and filtered. The leaf then squashed on mortar and pestle. Then this extract is diluted to 50%, 75% and 100% concentrations.

Preparing root tip squashes:

- Cut the tip 5-8 mm from tip of the fleshy sprouted root. Discarded the rest of the root.
- Placed the cut tips on a Petri plate containing alcohol for arresting the division.
- Took a root tip from Petri plate and washed it with water and transferred it to a clean microscopic slide.
- Added 2-3 drops of 6N HCl and waited for a minute, washed out the HCl from the root tip using water.
- Added 2-3 drops of toluidine stain and waited for two minutes. Then removed the excess of stain using blotting paper carefully and washed with water.
- Placed a drop of glycerin over it and covered it with a cover slip.
- Smashed the preparation using right thump over the cover slip to spread the cells evenly.
- Then observed the slide under the microscope.

The same procedure was repeated for the onion root treated with plant extract.

Examination:

The slides were first examined under low power (10 x) then in high power (40 x). One field was selected and counted the number of prophase, metaphase, anaphase, telophase and undivided cells. Likewise count is taken on cells treated with different concentrations. By taking the average of total number of cells counted prophase, metaphase, anaphase, telophase and undivided cells. Mitotic index was calculated by using the formula,

$$\textbf{M.I = (number of cells in mitosis/total number of cells) × 100}$$

SL. NO.	Dividing cells	Non dividing cells	Total cells counted	M.I%
1	20	22	42	47.6
2	19	32	51	37.2
3	20	37	57	35.0
Average	19.6	30.3	30.3	39.2

Table 1:- Number of cells in each stage of mitosis in untreated cells

Concentrations	Dividing cells	Non dividing cells	Total cells counted	M.I%
50%	12	34	46	26.08
75%	12	37	49	24.48
100%	9	36	45	20

Table 2:- Number of cells in mitosis treated with leaf extract of *Azadirachta indica:*

Table 3:- Number of cells in mitosis treated with root extract of *Curcuma longa:*

Concentrations	Dividing cells	Non dividing cell	Total cells counted	M.I%
50%	14	36	50	28
75%	10	38	48	20.83
100%	10	39	49	20.4

Figure 1: Mitotic index of control and different concentrations of neem extract.

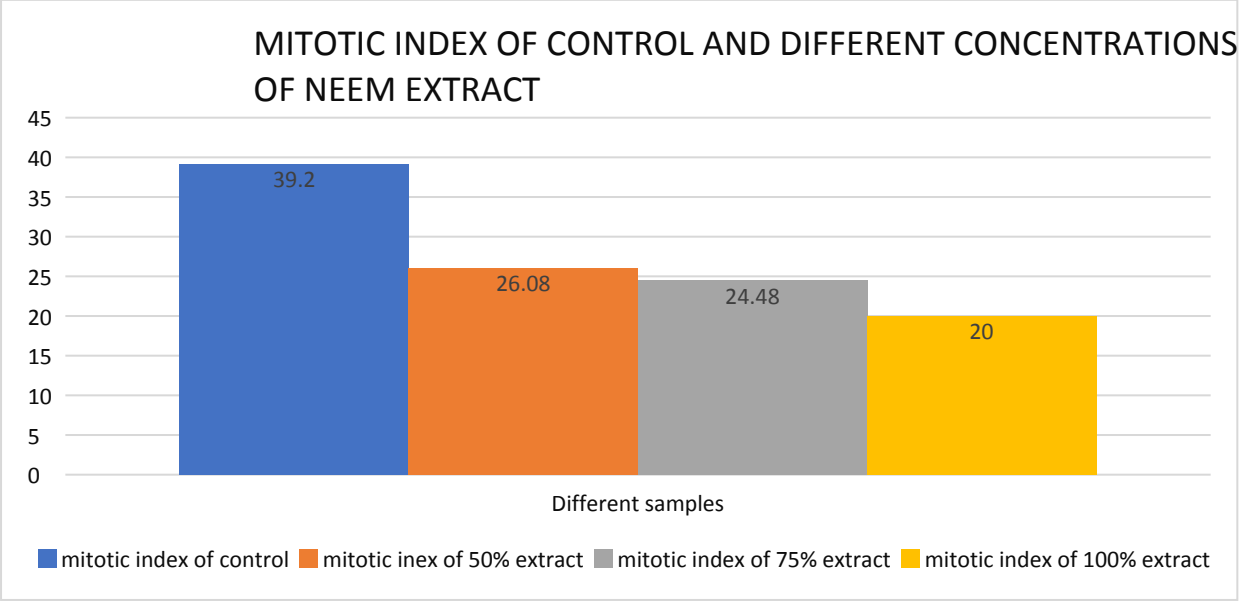
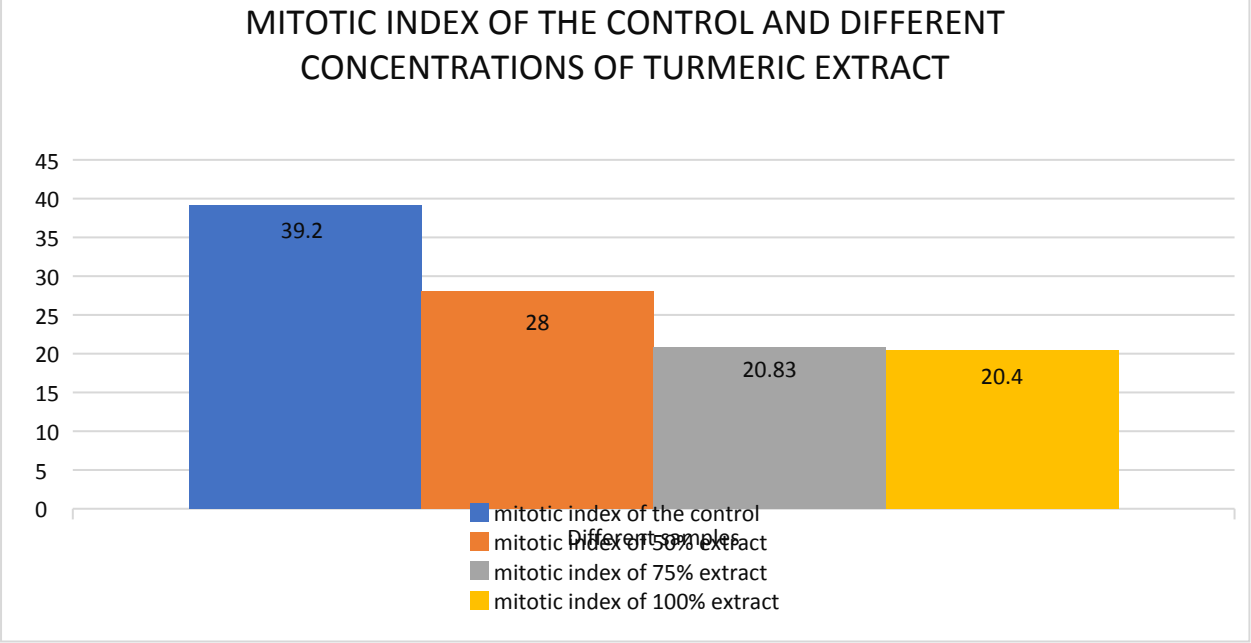
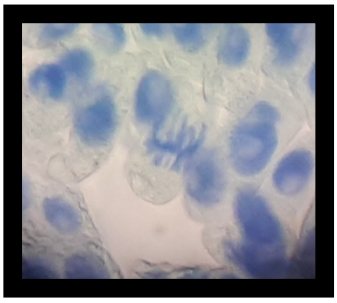


Figure 2: Mitotic index of control and different concentrations of turmeric extract.



DIAGRAMS SHOWING MITOTIC STAGES OF UNTREATED AND TREATED CELLS



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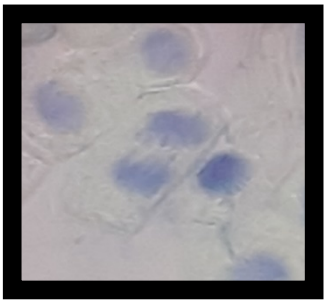

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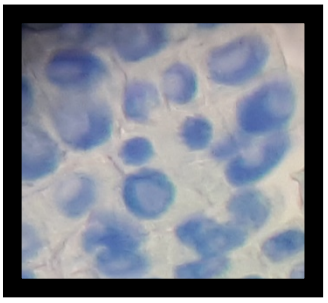

C

DIFFERENT STAGES OF MITOSIS: **A.** Metaphase **B.** Anaphase **C.** Telophase

The cytological observations from treated root tip cells revealed that the extracts of *Azadirachta indica* and *Curcuma longa* had a strong mitodepressive effect on *Allium cepa* roots. Mitotic index and number of cells in different stages of cell division after treating the root tips of onion with different concentrations of leaf extracts are shown in the Table 2, 3.Depression of mitotic index increased with an increase of concentration. Abnormalities like ring chromosomes and chromosome bridges were also observed. Depression of cell division was seen obviously in the highest concentration used where the mitotic index reached not more than 26.08% in *Azadirachta indica* and 28% in *Curcuma longa* compared to 39.2% in the control. In higher doses it was found to have more deleterious effects.

In the present investigation both the plant extracts showed mitodepressive effect by causing disturbances in microtubule polymerization by binding to the protein tubulin, which leads to microtubule degradation. This disrupts mitosis and leads to cell death because microtubules are a component of cytoskeleton found throughout the cell, cytoplasm which is important for the formation of spindle fibres during the process of mitosis is without microtubule and the cell could not divide. This could be very beneficial in the treatment of cancer because plant extract could possibly stop cancerous cells from dividing and can be used as natural source of anti-cancer drug.

DISCUSSION

Present study was conducted to know the effect of two medicinal plants on the mitotic index of *allium cepa* (onion) root tip. Onion root tip was treated with extracts of *Curcuma longa* and *Azadirachta indica*.

Soudamini *et al*. reported that some spices (turmeric) may ameliorate the effects of environmental mutagens, especially those present in food. In the present study curcumin exhibited clastogenic activity in *Allium cepa* L. root meristem cells in a dose dependent manner. Curcumin has been reported to induce a significant increase in the frequency of chromosomal aberrations in Chinese hamster ovary cells. Holy (2002) reported micronucleus induction in combination with centrosome immuno labeling in response to curcumin in MCF-7 cells, indicating that most micronuclei are centromere positive and caused significant abnormalities in spindle and centrosome organization, confirming that curcumin plays a significant role in spindle disruption, which raises the possibility that curcumin may promote genetic instability under some circumstances. Ishidate (1984) observed that curcumin was clastogenic at 30 μg/mL and Araujo *et al*. reported that curcumin at 10μg/mL was clastogenic in mammalian cell culture.

Antunes (2001) also reported the potentiating effect of curcumin on doxorubicin-induced chromosome damage in Chinese hamster ovary cells. It has reported that Curcumin not only failed to prevent single strand DNA breaks by hydrogen peroxide, but also caused DNA damage.

Curcumin was also reported to cause oxidative damage in rat hepatocytes and human erythrocytes. Additionally, Curcumin did not protect against DNA strand breaks in human lymphocytes and gastric mucosal cells.

Previous studies showed that Curcumin (20μg/mL) had antimutagenic potential against sodium azide-induced chromosomal aberrations in *Allium cepa* L. root meristem cells. In addition, it showed that there was mild cytotoxicity and lower MI scores in all the curcumin treated groups. Previous studies that reported the antioxidant curcumin could become a pro-oxidant agent in accordance with the redox state of the biological environment may support the present study's observation of the clastogenic activity of curcumin. Cao *et al*. reported that higher concentrations of curcumin caused oxidative stress and DNA damage.

The reductions in the MI observed in the present study indicate that curcumin exhibited cytotoxicity activity in the *Allium cepa* L. test system. Adams *et al*. concluded that the curcumin analogue exhibits a high degree of toxicity under in vitro conditions. Kuttan *et al*. observed its cytotoxic effect in CHO cells within 30 min at room temperature. The production of cells with extensive chromosomal abnormalities after curcumin treatment suggests that at least some of the cytostatic effect of this phytochemical is due to its ability to disrupt normal mitosis.

The use of biological active substances from neem for the control of agriculture pests, has been undertaken in different parts of the world. In this study neem leaf extract suppressed the mitotic activity of allium root meristems after 24 hr treatment with all concentrations.

Many other investigations shows that the reduction in the cell division activity could be due to change in the duration of mitotic cycle. Van't Hoff (1968) suggested that, the inhibition to mitotic activity by chemical compound is due to increase in the G2 period

Polyploidy and bi- and multinucleated cells were observed in various other works, (Sudharsan and Reddy, 1971). This may be due to inhibition of spindle mechanism and suppression of phargmoplast formation in early telophase respectively (Shehab *et al*. 1978). Disturbed metaphase, anaphase and telophase might observe due to the disturbance of the spindle apparatus.

In this study mitotic index got lowered in treated cells when compared to untreated cells as the extracts inhibited mitotic activity that caused disturbances in microtubule polymerization by binding to the protein tubulin, which lead to microtubule degradation. This disrupted mitosis by inhibiting spindle formation because microtubules are essential for the formation of spindle fibers and lead to cell death. This could be very beneficial in the treatment of cancer because these plant extracts could possibly stop cancerous cells from dividing and can be used as natural source of anticancer drugs (Bharathi *et.al*.,2006).

CONCLUSION

The current study helped to evaluate the anti-mitotic effect of two medicinal plants *Azadirachta indica* and *Curcuma longa*. Onion root tips were treated with leaf and root extract of these plants respectively in different concentrations. The mitotic activity of onion root tip cells were noted by counting the number of cells in each stage of mitosis and mitotic index was calculated and compared the values with untreated onion root tip as control.

Results obtained showed that plant extracts had an effect on mitosis. It lowers the mitotic index and percentage of cells going through different stages of mitosis. Also the percentages of dividing cells decreased with an increase in the concentration of extracts. Therefore cells treated with plant extracts were able to enter into prophase but since their microtubules have become weak, they were unable to advance to later stages of mitosis.

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A SURVEY ON THE PREVALENCE AND PATTERNS OF DIABETES MELLITUS IN KOCHI CITY

Dissertation submitted to the

Sacred Heart College (Autonomous) Thevara

In Partial fulfillment of the Degree of Bachelor of Science in Zoology

By

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DEPARTMENT OF ZOOLOGY

SACRED HEART COLLEGE (AUTONOMOUS)

THEVARA, COCHIN – 13

2016-2019

A STUDY OF BUTTERFLY DIVERSITY IN UDAYAMPEROOR

Dissertation submitted to

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Kochi-13, Kerala, India

In partial fulfillment of the requirements for the Degree of Bachelor of Science in Zoology

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Herbal plants as an Ant repellent

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Catharanthus roseus AND Euphorbia hirta

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DEPARTMENT OF ZOOLOGY

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THEVARA, COCHIN – 13

2016-2019

QUANTITATIVE ANALYSIS ON THE ANTIBACTERIAL EFFECT OF THE COMMON DISHWASH LIQUIDS

Dissertation submitted to the

Sacred Heart College (Autonomous) Thevara

In Partial fulfillment of the Degree of Bachelor of Science in Zoology

BY

SANDRA GEORGE

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2016-2019

CERTIFICATE

This is to certify that the project report entitled "**Quantitative Analysis on the Antibacterial Effect of the Common Dishwash Liquids**" is an authentic record of research work carried out by **SANDRA GEORGE**, Reg No. **16UZOO4843** in partial fulfillment of the requirements of the Degree of Bachelor of Science of Sacred Heart College, Thevara (Autonomous) during the year 2016-19.

Dr. M.K. Raju

Head of the Department

Sacred Heart College

Thevara

EXAMINERS:

Place:

Date:

CERTIFICATE BY THE GUIDE

This is to certify that the contents of this work entitled "**Quantitative Analysis on the Antibacterial Effect of the Common Dishwash Liquids"** is the original research work done by **SANDRA GEORGE** under my supervision of guidance.

I further certify that the work has not been submitted either partly or fully to any other university or institution for the award of any degree or diploma.

Dr. Smitha .S.
Associate professor
Department of Zoology
Sacred Heart College, Thevara

Place: Thevara
Date:

DECLARATION

I hereby declare that this project entitled "**Quantitative Analysis on the Antibacterial Effect of the Common Dishwash Liquids"** is a genuine record of research work done by me during the academic year 2018-19 in partial fulfillment of the requirement of Bachelor of Science degree under the guidance of Dr.Smitha .S, Associate professor, Department of Zoology, Sacred Heart College, Thevara and has not been submitted either partly or fully to any other university or institution for the award of any degree or diploma.

I further declare that the results presented in this work and considerations made therein, contribute in general to the advancement of knowledge in science.

SANDRA GEORGE

Place **:**

Date :

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I am grateful to the Head of the Department of Zoology, S.H. College, Thevara for his sincere help and encouragement throughout the course of this project work.

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SANDRA GEORGE

Place **:**

Date :

CONTENTS

ABSTRACT:

One of the main causes of bacterial growth in kitchens are kitchen sponges, improperly washed chopping boards and dishcloths. People usually do not wash the cutting board immediately after use. The surface of the cutting board can harbour large number of bacteria that may be pathogenic. Many people are not aware of the bacteria these surfaces can carry.

Cutting boards are commonly perceived as important fomites in cross-contamination of foods with agents such as *Salmonella* sp. Meat is highly nutritious, so it promotes microbial growth to a greater extent. The present work was carried out to analyse the antibacterial effect of commonly used dishwash liquids such as Vim, Lux(sunlight), Lux(lemon), handmade liquid and pril.

The method employed was Standard plate count (SPC). Meat was wiped on the surface of cutting board and kept for 30 minutes. The dishwash liquids were applied on the board and kept for ten minutes. Sample for analysis was collected using a sterile swab and SPC was performed. Application of water was taken as control. The bacterial colonies obtained were counted and recorded. From the observations and calculations, Vim was found to be most effective dish wash liquid followed by Lux (sunlight), Lux(lemon), handmade liquid and pril.

INTRODUCTION:

There are many ways that bacteria can grow and cross-contaminate things throughout homes.One of the main causes of bacterial growth in kitchens are kitchen sponges, improperly washed chopping boards and dishcloths. People usually do not wash the cutting board immediately after use.The surface of the cutting board can harbour large number of bacteria that may be pathogenic. Many people are not aware of the bacteria these surfaces can carry. Food poisoning is a common occurrence in the United States with 5.5-6.5 million cases reported per year (Nielsen, 2002). Food poisoning is often a result of cross contamination in the kitchen. The most common types of bacteria found on cutting board surface after use are the following: *Escherichia coli;Pseudomonas aeruginosa* and *Staphylococcus*. Diseases can be spread by repeated use of cutting boards without washing it after use. There is a chance of the food being chopped to get contaminated. It is reported in the journal, *Infection Control and Hospital Epidemiology*that"Chopping boards are a dangerous source of cross-contamination if not cleaned, stored and used properly, and replaced due to wear and tear with age". This work throws light on the relevance of washing cutting board with commonly used dishwashing liquids.

The failure to effectively remove bacteria from food contact surfaces can have serious implications in the transmission of food borne disease. At home a major concern is the transmission of food borne pathogens by cross-contamination of foods via food contact surfaces, particularly chopping boards, which is found to be one of the top five sites most contaminated with heterotrophic bacteria in the kitchen (Josephson et al., 1997). Other studies also found cross-contamination via domestic hand and food contact surfaces to be a significant contribution to cross-infection and there is a constant risk of microbial transfer from these surfaces (Bloomfield and Scott, 1997; Cogan *et al.,* 1999; Gorman et al., 2002). Many antibacterial products have been developed to provide fast and effective cleaning to food preparation areas, replacing the traditional two-step detergent and rinse cleaning method. Many of these products such as sprays and wipes are cleaners containing an antibacterial agent and the instructions do not advocate rinsing after their use, despite evidence showing that rinsing is a vital step in the cleaning of domestic food contact surfaces (Cogan et al., 1999; Rusin et al., 1998; Kusumaningrum et al., 2002). A number of investigations demonstrated effectiveness of antibacterial products on food pathogens. For example, antibacterial dishwashing liquid was effective

in reducing pathogens in laboratory suspension test although not in used sponges (Kusumaningrum et al., 2002); cloths treated with quaternary ammonium compounds reduced cross contamination significantly (Scott and Bloomfield, 1993).

According to the Department of Health, the highest occurred cause for food borne outbreaks in Taiwan is cross-contamination, which mostly occurs on the surfaces of kitchen utensils (Bureau of Food Safety, 2008). The United State (FDA, 2008) and Europe (Beumer&Kusumanigrum, 2003) also emphasize that avoiding cross-contamination is the key procedure to prevent the occurrence of food borne illnesses. Pathogenic bacteria could be transferred between raw materials, cloth, hands, and food contact surfaces to create cross-contamination (de Boer &Hahné, 1990; Scott & Bloomfield, 1990). Currently, the most common practice to eliminate bacteria on the food contact surfaces is washing with detergent at elevated temperatures.

Rusin*et al.* (1998) and Josephson *et al.* (1997) showed that even effective antimicrobial agents will not reduce levels of bacteria if not incorporated into an appropriate cleaning regime. In addition, the public may not be as vigilant with the cleaning and decontamination of food contact surfaces as they could be (Jay *et al.*, 1999). The speed and relative ease of cleaning provided by antibacterial sprays and wipes could be favored above that of the proven detergent rinse method of cleaning. It is therefore important to establish how effective such products are at inhibiting bacteria and protecting the public from cross-contamination. The cutting board is of interest as it poses constant risk of infection in the domestic environment (Rusin*et al.*, 1998). This study investigates the effectiveness of domestic antibacterial wipes and sprays in preventing cross-contamination in a household using a single cutting board initially for the preparation of raw meat followed some time later by the preparation of high-risk ready to eat food. The condition under investigation is the products effectiveness when used up to 2 h after the preparation of contaminated food but immediately before the preparation of ready to eat food.

A survey of more than 2,000 people across the UK, commissioned by Sainsbury's Home, found 40 per cent of people are putting themselves at risk by using the same chopping board for meat and vegetables.Raw meat, particularly raw chicken, can leave traces of salmonella and campylobacter that cause food poisoning. These germs can contaminate any food that is prepared on the same chopping board.

Background of the study:

Bacteria

Bacteria are "any of a large group of very small one-celled organisms that reproduce by fission or by forming spores. Some kinds can cause disease, while others are active in fermentation." The American Heritage Student Dictionary, 1994 Edition.

Bacteria are one-celled organisms that are classified as prokaryotes, meaning they have no nucleus. They are approximately 2-3 microns in diameter (a micron is 1/24,500 of an inch or 0.001 millimeters.)

Antoni van Leeuwenhoek made microscopes in Holland during the late 17th century. He was the first person to study bacteria and worked hundreds of hours to make ground glass lenses for his microscopes. Leeuwenhoek was the first person to discover bacteria as well as study them. Robert Koch proved that bacteria are disease causing.

Dishwashing Liquid:

Dishwashing liquid, known as dishwashing soap, dish detergent and dish soap, is a detergent used to assist in dishwashing. It is usually a highly-foaming mixture of surfactants with low skin irritation, and is primarily used for hand washing of glasses, plates, cutlery, and cooking utensils in a sink or bowl.

The main ingredient is water; the main active ingredients are detergents. Detergents are used, rather than soaps, because they don't react with any hardness in the water to form soap scum. There are other thickening and stabilizing agents. Other ingredients may include surfactants, hydrograph, salts, preservatives, fragrances, and dyes. Many dishwashing liquids contain perfume which can cause irritant or allergic contact dermatitis.

Surfactants remove grease and stuck food particles. They may also provide foam.

Some dishwashing products contain phosphates in detergent. Phosphate makes dishes cleaner but can also cause harmful algal bloom as the wastewater goes back to the natural environment. Because of this, it is banned as a component in many places.

In 2010, the United States FDA raised health concerns over triclosan, an antibacterial substance used in some dish liquids. Elsewhere, triclosan has been found to create problems at wastewater treatment plants, whereby it can "sabotage some sludge-processing microbes and promote drug resistance in others". The United States FDA has found that triclosan provides no health benefits over soap and water. As of 2014, at least one state within the United States has banned triclosan in dishwashing liquids.

The types of ingredients in dish-washing detergent can be of two categories: active ingredients and inactive ingredients. Active ingredients are the ingredients that actually do the work that the product is intended to do. In dish-washing detergent, the active ingredient is what kills the bacteria on your grimy dishes.

Active Ingredients -Triclosan is the active ingredient in dish-washing detergent (as well as anti-bacterial hand soaps) whose primary purpose is to stop the growth of bacteria, mildew and fungi. Triclosan is present in concentrations of up to 2 percent in dish-washing and hand soaps. Generally, however, significantly less triclosan is used in these products.. No long-term health risks are associated with the use of triclosan in dish-washing detergents.

Inactive Ingredients -A wide range of inactive ingredients are used in dishwashing detergents. Water is the most common of these inactive ingredients. All liquid dishwashing detergents have a high percentage of water in their composition, regardless of brand. Other common ingredients include coloring dyes, fragrances, preservatives, sodium chloride (to help control the thickness of the detergent), lauramidopropylamine oxide (as a foaming agent) and SD alcohol 3-A (to help control thickness and clarity).

Product Details

VIM Liquid is created with real lime juice and has superior degreasing abilities leaving dishes sparkling and shiny. Ingredients- Sodium LAS, Disodium EDTA, SLES, Concentrated Lime Juice, CI 19140, CI 42051, Water.

LUX (SUNLIGHT):Sodium dodecylbenzene sulfonate 20 - 30% , Sodium lauryl ether sulfate 10% , Sodium xylene sulfonate < 5% , Ethyl alcohol< 3% , Cocamidopropyl betaine < 3% .

LUX (LEMON)dishwash's formulation cuts through tough grease with rich, thick suds to get your dishes sparkling clean while being gentle on your hands. Ingredients- 15-30% Anionic Surfactants, Benzisothiazolinone, Methylchloroisothiazolinone, Methylsothiazolinone, Perfume, Limonene, Linalool, Citral.

HANDMADE LIQUID 2/3 cup Sal Suds.1 and 1/3 cup distilled water.40 drops lemon or grapefruit essential oil (or essential oil of choice)1TBSP washing soda, 1TBSP table or kosher salt and 3 TBSP hot water in large pot, dish soap dispenser.

PRIL Multi Power Dishwashing Liquid delivers the best performance in removing baked-on stains. It is formulated with a multi power formula that fights against grease and crust. It has a thick texture yet dissolves quickly in water, thereby providing you with an excellent cleaning performance. It has a pleasant smell that refreshes your senses.

The compounds functions by denaturing cell activity and interfering with microbial metabolism.Thus it is routine practice to wash cutting boards with dishwash liquid in order to remove some potentially harmful bacteria.

Chopping boards:

Chopping boards are an essential item in any kitchen. They are used to cut meat, vegetables, fruits or bread. Chopping boards need to be kept clean in order for them to be used safely. It is best to separate the chopping board used for cutting meat, from boards used for cooked food and food that is to be eaten immediately like bread, fruit or fresh vegetable salads. This is to prevent cross contamination.
Both wooden and plastic chopping boards are safe to be used as long as they are properly cleaned. However it is believed that wooden chopping boards are safer because any bacteria on the surface of the board will be pulled into the wood by capillary action. The bacteria will be unable to multiply once it is inside the wood and eventually they will die.Plastic boards are not porous and therefore have surfaces that are easier to clean. However, under normal usage, scars will be left on the board's surface and this creates a place for the bacteria to grow and multiply. Therefore old and scarred plastic chopping boards should be disposed of as soon as signs of damage/scarring appears. Dr Lisa Ackerley, The Hygiene Doctor, has revealed the common mistakes with chopping board — including washing them in the wrong way and not replacing them regularly.

Statement of the problem:

The study sought to compare the effectiveness of the different brands of commercialized dishwashing liquid as to elimination of food borne bacteria.

Specifically the study was conducted to answer the following questions:-

- Which brand is the best to use in the elimination of food bornebacteria?
- Which brand works well with beef meat?

Research Design:

The study employs the inferential design in assessing the effect of different commercialized dishwashing liquid. This study uses 5 different brands of dishwashing liquid and also water as control.

The Complete Randomized Design (CFD) was used (randomly chose 5 different dishwashing liquid and below mentioned ones were chosen:-

- Treatment 1 _ _ _ _ _ _Pril
- Treatment 2 _ _ _ _ _ _ Vim
- Treatment 3 _ _ _ _ _ _Handmade liquid
- Treatment 4 _ _ _ _ _ _ Lux(Lemon)
- Treatment 5 _ _ _ _ _ _ Lux(Sunlight)
- Treatment 6 _ _ _ _ _ _ Water (*as control)

Locale of the study:

The study was conducted in the PG Laboratory of Department Of Zoology at SH College,

AIM:

To determine the effect of commonly used dishwash liquids on bacteria contaminating the cutting board.

OBJECTIVES:

- To collect data on different brands of dishwash liquid used by common people in the kitchen.
- Qualitative analysis in the laboratory by standard plate count method.

REVIEW OF LITERATURE:

According to the Department of Health, the highest occurred cause for foodborne outbreaks in Taiwan is cross-contamination, which mostly occurs on the surfaces of kitchen utensils (Bureau of Food Safety, 2008). The United State (FDA, 2008) and Europe (Beumer & Kusumaningrum, 2003) also emphasize that avoiding cross-contamination is the key procedure to prevent the occurrence of foodborne illnesses. Pathogenic bacteria could be transferred between raw materials, cloth, hands, and food contact surfaces to create cross-contamination (de Boer &Hahné, 1990; Scott & Bloomfield, 1990). Currently, the most common practice to eliminate bacteria on the food contact surfaces is washing with detergent at elevated temperatures.

Outbreaks of food poisoning frequently occur as a result of cross-contamination (Olsen *et al*., 2000). Dishcloths and sponges were recognized as a potential source for spreading microorganisms and it was observed that bacteria persisted in these vehicles (Josephson *et al.,* 1997; Rusin*et al*., 1998). Studies of the domestic environment by Finch et al. (1978), Scott et al. (1982), Speirs *et al*. (1995), Josephson *et al*. (1997) and Rusin*et al*. (1998) indicate that micro-organisms, including some potentially pathogenic species, are commonly found in all areas of the home environment. The failure to effectively remove bacteria from food contact surfaces can have serious implications in the transmission of foodborne disease.At home a major concern is the transmission of foodborne pathogens by cross-contamination of foods via food contact surfaces, particularly chopping boards, which is found to be one of the top five sites most contaminated with heterotrophic bacteria in the kitchen (Josephson *et al*., 1997). Other studies also found cross-contamination via domestic hand and food contact surfaces to be a significant contribution to cross-infection and there is a constant risk of microbial transfer from these surfaces (Bloomfield and Scott, 1997; Cogan *et al*., 1999; Gorman *et al*., 2002). Many antibacterial products have been developed to provide fast and effective cleaning to food preparation areas, replacing the traditional two-step detergent and rinse cleaning method. Many of these products such as sprays and wipes are cleaners containing an antibacterial agent and the instructions do not advocate rinsing after their use, despite evidence showing that rinsing is a vital step in the cleaning of domestic food contact surfaces (Cogan *et al*., 1999; Rusin et al., 1998; Kusumaningrum*et al*., 2002). A

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T.A. Cogan *et al* (2001) conducted a study to quantify the transmission of *Salmonella* and *Campylobacterto* on hands, cloths, and hand- and food-contact surfaces during the preparation of raw poultry in domestic kitchens and to examine the impact on numbers of these bacteria of detergent-based cleaning alone.Rinsing, as part of the cleaning process, is a critical step in achieving hygiene in the kitchen. However, to achieve completely hygienic surfaces, the use of an antimicrobial agent may be necessary.

Elaine *et al* (2004) evaluated the effect of antibacterial cleaning and handwashing products for consumers on the occurrence of infectious disease symptoms in households.

Kusumaningram *et al* (2001) investigated the effects of an antibacterial dishwashing liquid on *Escherichia coli*, *Salmonella enteritidis*, *Staphylococcus aureus*, and *Bacillus cereus* in a modified suspension test in used sponges with and without food

residues under laboratory conditions. The results of this study demonstrate that the antibacterial dishwashing liquid was effective in reducing pathogens in the suspension test but not in the used sponges. This finding determined the efficacy of antibacterial products.

Peter Nielsen *et al* (2002) evaluated the effect of adding commercial liquid hand dishwashing detergents to kitchen sponges to control microbial growth. Surviving microorganisms were then quantitated using either the spiral or pour plate method. Untreated sponges showed stasis or slightly increased bacterial populations after the incubation period in the absence of NFDM. Statistically significant reductions were observed for clean sponges and sponges soiled with NFDM when detergents making "antibacterial sponge" claims were added to the inoculated sponges.

Ozlem and Feryal (2005) studied the presence of various kinds of microorganisms in kitchen sponges. Additionally effects of regular dish washing liquid on *Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 13311 were investigated under laboratory conditions in kitchen sponges with or without food residues. Enriquez *et al.* (1997) isolated and identified 23 different bacterial species from 140 sponges, and 13 bacterial species from 56 dishcloths from US homes. The most common bacteria were *Enterobacteriacae* and *Pseudomonas sp*. *Salmonella sp*. were identified in 15% of sponges and 14% of dishcloths. A UK study also found 84% of dishcloth samples contaminated with *Listeria sp.* (Duggan and Phillips 1998). Hilton and Austin (2000) sampled 100 dishcloths and sponges from domestic kitchens and isolated S. aureus from 4% of spongetype materials, with counts ranging from 102 to 4 x 104 cfu/ml. The total viable count from all types ranged from 20 to 6 x 108 cfu/ml with a mean of 8.5 x 107 cfu/ml.

Many factors have been shown to influence bacterial survival and transfer between surfaces, including temperature (Williams *et al.*, 2005), bacterial type (Scott and Bloomfield, 1990; Rusin *et al.*, 2002), nature of surfaces (Chen *et al.*, 2001; Gill and Jones, 2002; Rusin et al., 2002), time lapsed post-inoculation (Scott and Bloomfield, 1990), moisture level (Gill and Jones, 2002; Williams et al., 2005) and inoculum size (Montville and Schaffner, 2003). To truly test the effectiveness of these products in domestic kitchens, it would be necessary to carry out an in-use study; this would take into account food debris, consortium of bacteria and not monocultures of laboratory species

and larger food contact surfaces. It would also be of interest to investigate how these products compare with cheaper disinfectants such as bleach.

Holah and Hall (2006) tested the role of dishwater, the washing fluid surrounding utensils, crockery and cutlery etc. washed-up in sinks or bowls, as a source of microbial cross-contamination has however, received little attention. Mattick *et al*. (2003b) found average dishwater aerobic plate counts (APC) of 1Æ3 · 105 CFU ml)1 from 52 private homes with 50% of APCs lying between 105 and 106 CFU ml)1 . *Enterobacteriaceae* counts averaged 7Æ1 · 104 CFU/ml)1 with 20% of counts lying between log10 5 and log10 6 and 20% lying between log10 4 and log10 5 CFU/ml)1 , respectively. Mosupye and von Holy (1999) recorded a mean APC of log10 4Æ0±1Æ2 (range 3Æ2–6Æ4) CFU/ml)1 from 18 dishwater samples from street vendors in Johannesburg, whilst Speirs *et al*. (1995) reported that 60% of washing-up bowls from 46 households had APCs of >log103 CFU/ml)1 .

TrondMoretro *et al* (2011)did a work to investigate if cutting boards containing the antimicrobial compound triclosan can reduce the viability of bacteria, thus acting as a hygiene barrier. Survival and growth of food pathogens and spoilage bacteria on two cutting boards without antimicrobials and a commercial cutting board containing triclosan were tested. No difference in bacterial counts on cutting boards without and with triclosan was found after exposure to naturally contaminated chicken filets for one hour. Repeated washing of the triclosan-containing cutting boards reduced the antibacterial effect, thus the amount of triclosan available on the surface seemed to be limited. In conclusion, using triclosan-containing cutting boards as a hygienic barrier may only work under certain conditions (low humidity, long exposure time, and clean conditions) and not against all genera of bacteria.

MATERIALS REQUIRED:

The following materials and equipment were used in the experimental part of the study :-

1) Water (as control)
2) 5 different brands of Dishwashing Liquid namely **Pril, Vim, Handmade liquid, Lux(Lemon), Lux (Sunlight).**
3) Culture media (agar medium).
4) Petri dishes
5) Test tubes
6) Conical flask
7) Spirit lamp
8) Marker
9) Chopping board
10) Sterilized swab
11) 1ml pipette
12) Distilled water

Figure 1. Materials used for the study

Figure 2. On top_Cutting board, On left_Samples prepared, On right_Meat taken to rub the board

METHODOLOGY:

1) **Sample collection-** The dishwash liquids used for the study were purchased. The batch numbers, expiry dates and the presence or absence of the manufacture seal was noted.

2) **Division of cutting board into sections** –The cutting board was divided into 6 different sections for each of the 6 different dishwashing liquid by using a permanent marker followed by labeling each sections with the corresponding names of the dishwashliquids.The board was labeled 'VIM','PRIL','HANDMADE','LUX(LEMON)','LUX (SUNLIGHT)','WATER' (*AS CONTROL)

3) **Rubbing of meat on the cutting board surface**– Wearing a gloves, a piece of meat was rubbed completely on the surface of the cutting board ,uniformly distributed in all sections. This setup was left for 30 minutes.Let the microbes and other food pathogens grow on this setup.

4) **Preparation of sample -** Boiling tubes were used to keep 10ml solution of the diluted dishwash liquid. 1ml of each of the solutions was dissolved in 9ml of sterile distilled water to give a stock solution. These stock solutions were then stored in refrigerator in well plugged tubes for future use.

5) **Preparation of saline solution in test tubes** – About 0.85g NaCl is required for 100ml distilled water. So 360ml saline solution was needed for filling 30 test tubes & 6 boiling tubes and this was prepared by dissolving 3.06g NaCl in 360ml distilled water.

6) **Preparation of nutrient Agar**– 1.3g of nutrient broth and 2g agar agar is required for 100ml distilled water. So to prepare 200ml culture media,2.6g nutrient broth and 4g

agar was added to a conical flask containing 200ml distilled water .If mixing does not occur properly, heat it gently for few minutes until it dissolves.

7) All materials that is required for this experimental project have to be sterilized under high pressure which is above 100^0C. Conical flask with nutrient solution, test tubes containing saline solution, swab, a beaker of distilled water, tip, Petri dishes etc. should be wrapped and carefully kept it for sterilization in the autoclave. Test tubes were autoclaved for sterilizationafter plugging the mouth of the tube with cotton plug.

Application of dishwashing liquid onto the cutting board – 6 different dishwashing liquids (already prepared in step4) were applied onto the corresponding 6 different sections on the cutting board, marked earlier. For instance, Vim is swabbed on the area marked as 'VIM' and so on. Keep it for 10 minute

8) **Serial dilution**–Before plating the selected samples were serially diluted so as to reduce the microbial concentration. Depending on the source of the sample used, there might be thousands or even millions of microorganisms per milliliter of sample. Thus to count we dilute the sample.

A serial dilution is a series of sequential dilutions used to reduce a dense culture of cells to more usable concentration. Each concentration will reduce the concentration of bacteria by specific amount. Label test tubes as 10^{-1} 10^{-2} 10^{-3} 10^{-4} up to 10^{-5} .Each tube contains 9ml of sterile saline solution. Prepare 10^{-1} dilution using appropriate volume of sample(1g/ml).This first tube has a 1:10 diltion of original sample. Using a sterile 1ml pipette,transfer 1ml out of the first tube(10^{-1}) and add this to the test tube labeled 10^{-2}.The testubes were shaken. Using a new sterile 1ml pipette, transfer 1ml from the 10^{-2} tube to 10^{-3} tube. Repeat the shaking procedure and continue up to 10^{-5}.

9) Later 1ml of diluted sample from each serially diluted test tube of the sample are collected and poured in each of the sterilized Petri plates. After that the sterilized nutrient agar medium having a bearable temperature is poured on to each inoculated petriplates. The nutrient agar solution is allowed to spread by gently moving the plate on the surface. The whole procedure is done in a sterilized surface and also in the

presence of a spirit lamp so as to avoid any bacterial contamination. Leave the agar to set.

10) **Incubation –** When the agar sets to solid mass each Petri plate is labeled for its dilution factor and the name of the dishwashing liquid and then the Petri dishes are placed in upright position in the incubator for 24 hours at 37^0C. During the incubation, each viable cell that has spread to a discrete position on the agar surface will grow and divide many times to form a visible colony of microorganisms. After te incubation period we can count the colonies to determine how many microbes were present in the original sample.

11) **Standard plate count using pour plate method** – One of the most fundamental microbiological techniques is plate counting which is used to determine the number of viable cells in a sample. Standard Plate Count (SPC) is a method that consists of growing bacteria in nutrient culture and counting colonies which develop. It can be used for all types of dishwashes and is generally used in this study in the examination of Comparative Analysis on the Antibacterial effect of different Dishwashing liquids on the cutting board surface.

The plates will have different number of colonies depending on the dilution of the sample. If there are too many colonies, it would be very difficult to count them. If there are only a small number of colonies it is easy to count them as the results are prone to error. As a compromise we always aim to count plates with between 30 and 300 colonies. We record our results noting the dilution and how many colonies there were on these plates.

To determine how many viable bacteria were present in the original sample,multiply the number of colonies obtained via plate count with its dilution factor. The process is repeated for an average estimation of microbial count of each sample. The results obtained were analyzed and tabulated to evaluate the effectiveness of dish wash liquid.

RESULTS:

Results of this investigation revealed that most of the assessed dishwash liquid have antibacterial activity, though to varying degrees as indicated by the standard plate count.When the efficiency of the common dishwash Liquid were compared using the SPC method, Vim was found to be most effective with a bacterial colony count of 7 x 10^3. And the least effective among the tested samples was Pril with a colony count of 16 x 10^3. Almost all the samples demonstrated antibacterial activity hence buttressing the information written on the labels that they destroy bacteria. This is due to the differences in the active ingredients and the type of formulations used.

The viable count obtained by using SPC method is recorded and tabulated below:-

NAME OF THE DISHWASH LIQUID	VIABLE COLONY COUNT(cfu/mL)	
	Trail1 at 10^{-3}dilution	Trail 2 at10^{-3}dilution
VIM	**7x 10^3**	**8 x 10^3**
LUX(SUNLIGHT)	**9 x 10^3**	**9 x 10^3**
LUX(LEMON)	**12 x 10^3**	**11 x 10^3**
HANDMADE	**14 x 10^3**	**12 x 10^3**
PRIL	**15 x 10^3**	**16 x 10^3**
WATER(*AS CONTROL)	**23 x 10^3**	**25 x 10^3**

Table 1 .Viable colony count obtained for each dishwash liquid used.

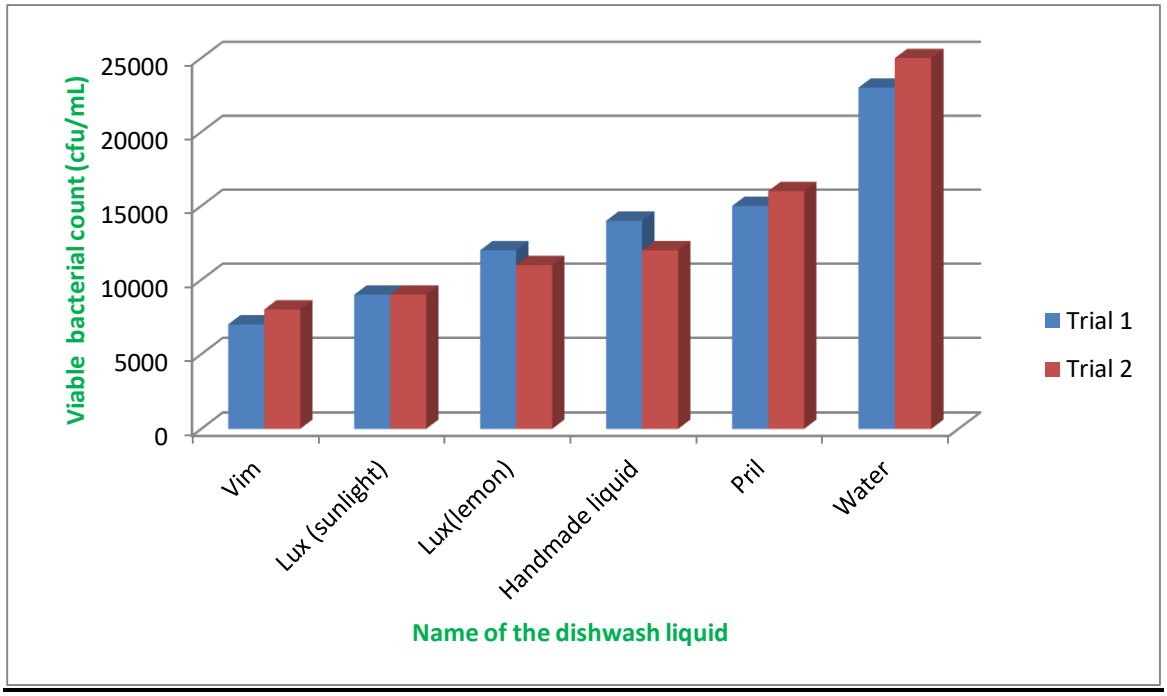

Chart 1.Antibacterial activity of different dishwash liquids in decreasing order of effectiveness.

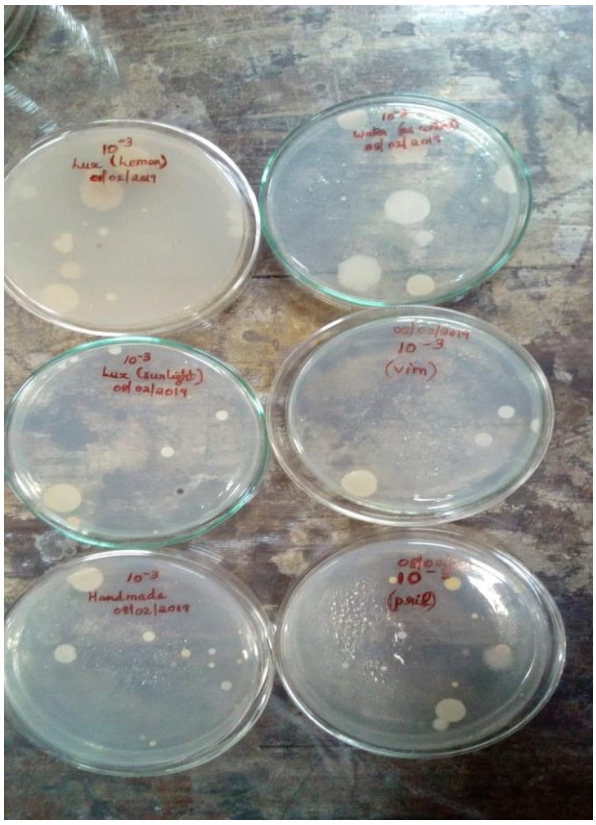

Figure 3. Petri plates showing bacterial colony obtained

As the table shows, the first comes Vim with 7 x 10^3cfu/mL and the second position for showing antibacterial activity was occupied by Lux (sunlight) with 9 x 10^3 cfu/mL, Lux (lemon) with 12 x 10^3cfu/mL, Handmade liquid with 14 x 10^3cfu/mL and the last position by Pril with 15 x 10^3cfu/mL. Water which was taken as control had 23 x 10^3cfu/mL.

This was then duplicated and the results were the following: Vim with 8 x 10^3cfu/mL, Lu(sunlight) with 9 x 10^3cfu/mL,Lux(lemon) with11 x 10^3cfu/mL, Handmade liquid with 12 x 10^3cfu/mL and Pril with 16 x 10^3cfu/mL. The control which was water , had 25 x 10^3cfu/mL.

The graph gives the inference that Vim shows least colony count, hence Vim is the best among the tested samples. And the largest colony count is shown by Pril which indicates that Pril is the least effective against antibacterial activity.

Generally the result of the study exhibited antibacterial activity. The lesser the number of bacteria means the more effective the sample is in the elimination of food borne pathogen.

DISCUSSIONS:

Studies of the domestic environment by Finch *et al*. (1978), Scott *et al.* (1982), Speirs *et al*. (1995), Josephson *et al*. (1997) and Rusin *et al*. (1998) indicate that micro-organisms, including some potentially pathogenic species, are commonly found in all areas of the home environment. The results of these studies indicate that wet sites, such as kitchen sink areas, draining chopping boards are most commonly associated with heavy contamination and the occurrence of potentially harmful species. The risk of cross-contamination during regular domestic cleaning is important since kitchen sponges and improperly washed cutting board were found to be potential vehicles of pathogens in domestic kitchens (Hilton and Austin, 2000) and pathogens were able to survive in kitchen sponges for at least weeks (Kusumaningrum *et a.l*, 2002).Enriquez *et al.* (1997) isolated and identified 23 different bacterial species from 140 sponges, and 13 bacterial species from 56 dishcloths from US homes. Hilton and Austin (2000) sampled 100 dishcloths and sponges from domestic kitchens and isolated S. *aureus* from 4% of spongetype materials. The total viable count from all types ranged from 20 to 6 x 108 cfu/ml.

Fisher and Phillips (2006), Fisher and Philips (2008) also suggested that the antibacterial effects of citrus essential oil were not uniform between bacteria.

Many factors have been shown to influence bacterial survival and transfer between surfaces, including temperature (Williams *et al*., 2005), bacterial type (Scott and Bloomfield, 1990; Rusin *et al.*, 2002), nature of surfaces (Chen *et al.*, 2001; Gill and Jones, 2002; Rusin et al., 2002), time lapsed post-inoculation (Scott and Bloomfield, 1990), moisture level (Gill and Jones, 2002; Williams *et al.*, 2005) and inoculum size (Montville and Schaffner, 2003). To truly test the effectiveness of these products in domestic kitchens, it would be necessary to carry out an in-use study; It would also be of interest to investigate how these products compare with cheaper disinfectants such as bleach.

Because the home environment harbors high levels of microbial contamination(Bloomfield .s and Scott .E,1997) and evidence shows that microorganisms can be readily transmitted between the ambient environment and humans, one might speculate that antibacterial products could result in a microbiologically cleaner

environment, which could translate to less human infection. On the other hand, the evidence to date has been primarily indirect or circumstantial.

In one study comparing ammonia, baking soda, borax, vinegar, a liquid dishwashing detergent, and bleach, only bleach was effective *against S. aureus, Salmonella typhi,* and *Escherichia coli* (Parnes .C, 1997). Absenteeism and respiratory infections were reduced with a comprehensive infection control program that included environmental disinfection in a specialized preschool, and upper respiratory infections were reduced in extended care facilities with a similar intervention(Morgan L, 2000). However, we could find no definitive evidence that use of antibacterial products for environmental cleaning reduced the risk for infections at home or restaurants, despite the fact that some investigators have found that use of detergent alone may actually seed the environment with more microorganisms (Josephson .K .L, 1997).

Any potential benefit of using antibacterial products for home hygiene must be weighed against the theoretical risk for antiseptic or antibiotic resistance. While there is no evidence that this has or will occur with the use of products containing small amounts of triclosan, in vitro tests have confirmed that some biological mechanisms allow such cross-resistance to occur in specific organism(Aiello .A .E and Lorson .E, 2003). Detergent has been shown to promote cell lysis of cell membranes, for example in molecular biology applications (Popowska et al., 1999); however, increasing the amount of detergent further (from 0.1% to 0.3%) was unlikely to significantly affect bacterial survival.

From this study, it is recommended to wash the cutting boards and plates with dishwash liquid instead of washing with plain water. Always wash utensils with dishwash liquid to prevent the occurrence of diseases particularly diarrhea. Bacteria can easily hide in scratches and crevices, ready to contaminate other food. Replacement of the scarred out cutting boards is recommended. Make sure all boards are kept in good condition. When preparing food it's important to use separate boards for raw and ready to eat foods. Further study could be conducted why the tested commercialized dishwash liquid are more effective in the elimination of bacteria.

CONCLUSIONS:

In the above result, it was stated that the commercialized dishwashing liquid are capable of eliminating bacteria.

- Among the 5 commercialized dishwashing liquid Vim has the greater potential in the elimination of bacteria.
- It was also found that Pril was the least effective.
- Further theses are more effective than using purely water.

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STUDY OF FINGERPRINTS IN RELATION TO GENDER AND BLOOD GROUP

Project submitted in partial fulfillment of the requirements for the Degree

Bachelor of Science in Zoology

BY

B SITHARA PARVEEN

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2018-2019

CERTIFICATE

This is to certify that the project work entitled "STUDY OF FINGERPRINTS IN RELATION TO GENDER AND BLOOD GROUP" is an authentic record of the work carried out by Ms.B SITHARA PARVEEN in the department as a part of B.Sc (Zoology) in practical with registration number 16UZOO4823 during 2016-2019.

Place: Thevara

Date:

Head of the Department

Department of Zoology

Sacred Heart College

Thevara

CERTIFICATE BY THE GUIDE

This is to certify that the contents of this work entitled "**STUDY OF FINGERPRINTS IN RELATION TO GENDER AND BLOOD GROUP"** is the original research work done by **B SITHARA PARVEEN** under my supervision of guidance.

I further certify that the work has not been submitted either partly or fully to any other university or institution for the award of any degree or diploma.

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Place: Thevara
Date:

CANDIDATE'S STATEMENT

I hereby declare that the work incorporated n the present dissertation is original and has not been submitted to any institution for the award of any diploma or degree.

I hereby declare that the results presented in the dissertation, consideration made therein, contribute in general to the advancement of knowledge of science '

Place: Thevara Signature of Candidate

Date:

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B SITHARA PARVEEN

CONTENTS

PARTICULARS

A COMPARATIVE STUDY ON FISH DIVERSITY OF MANGROVE ECOSYSTEM IN KUMBALANGHI VILLAGE

Dissertation submitted to

Sacred Heart College (Autonomous) Thevara

In Partial fulfilment of the Degree of Bachelor of Science in Zoology

By

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