بسم الله
الرحمن
الرحيم

Novel Formula

as

Mosquito Larvicides

Presented By
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Alexandria University

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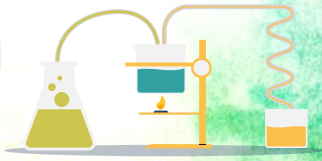
Introduction

CHAPTER ONE



INTRODUCTION

Vector-borne diseases account for more than 17% of all infections, causing more than 1 million deaths annually

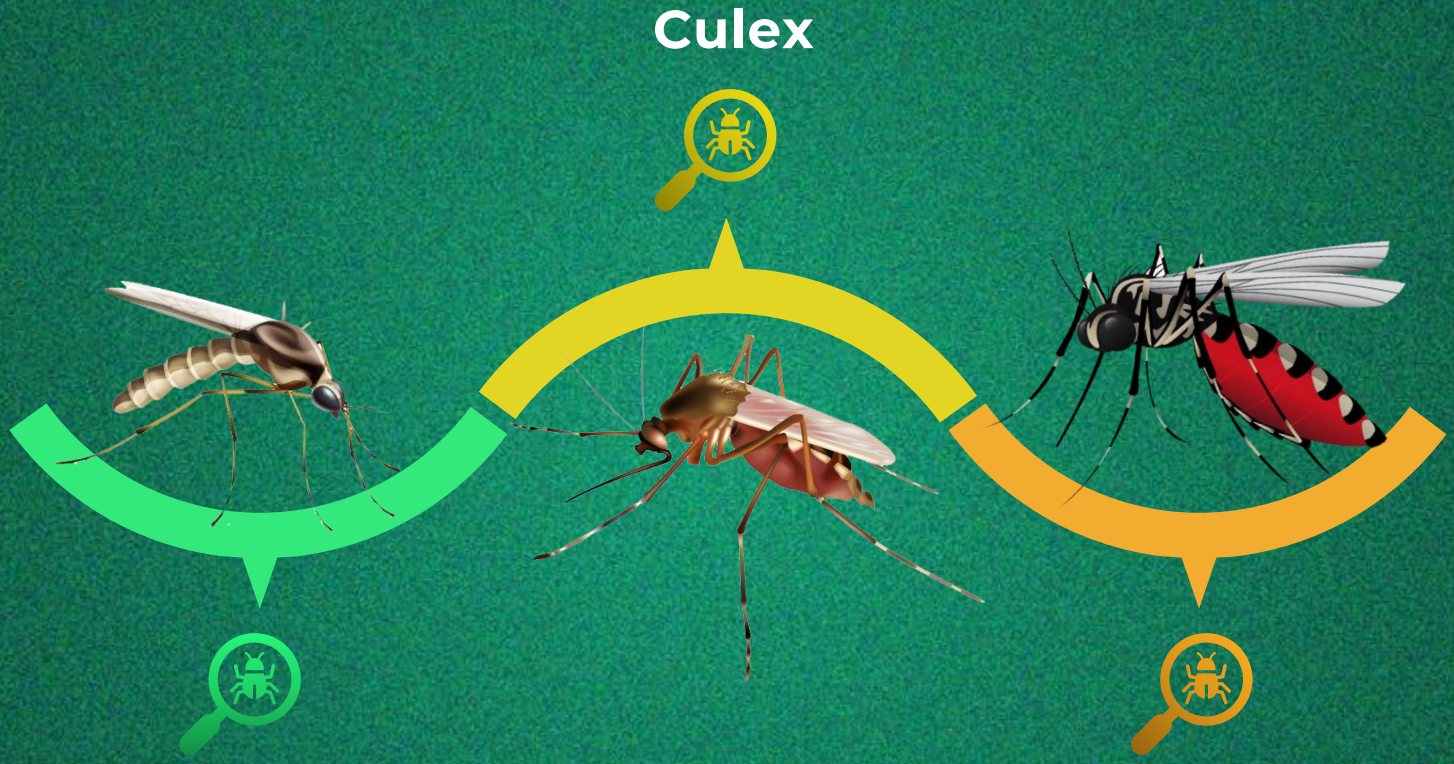
Mosquitoes are responsible for the transmission of many medically important pathogens and parasites such as:

- **Viruses**
- **Bacteria**
- **Protozoans**
- **Nematodes**

Which Cause Serious Diseases

INTRODUCTION

Several Mosquito Species Belonging to Genera



Are Vectors for the Pathogens of Various Diseases such as:

INTRODUCTION

Several Mosquito Species Belonging to Genera

Are Vectors for the Pathogens of Various Diseases such as:

01 **Malaria**

02 **Dengue**

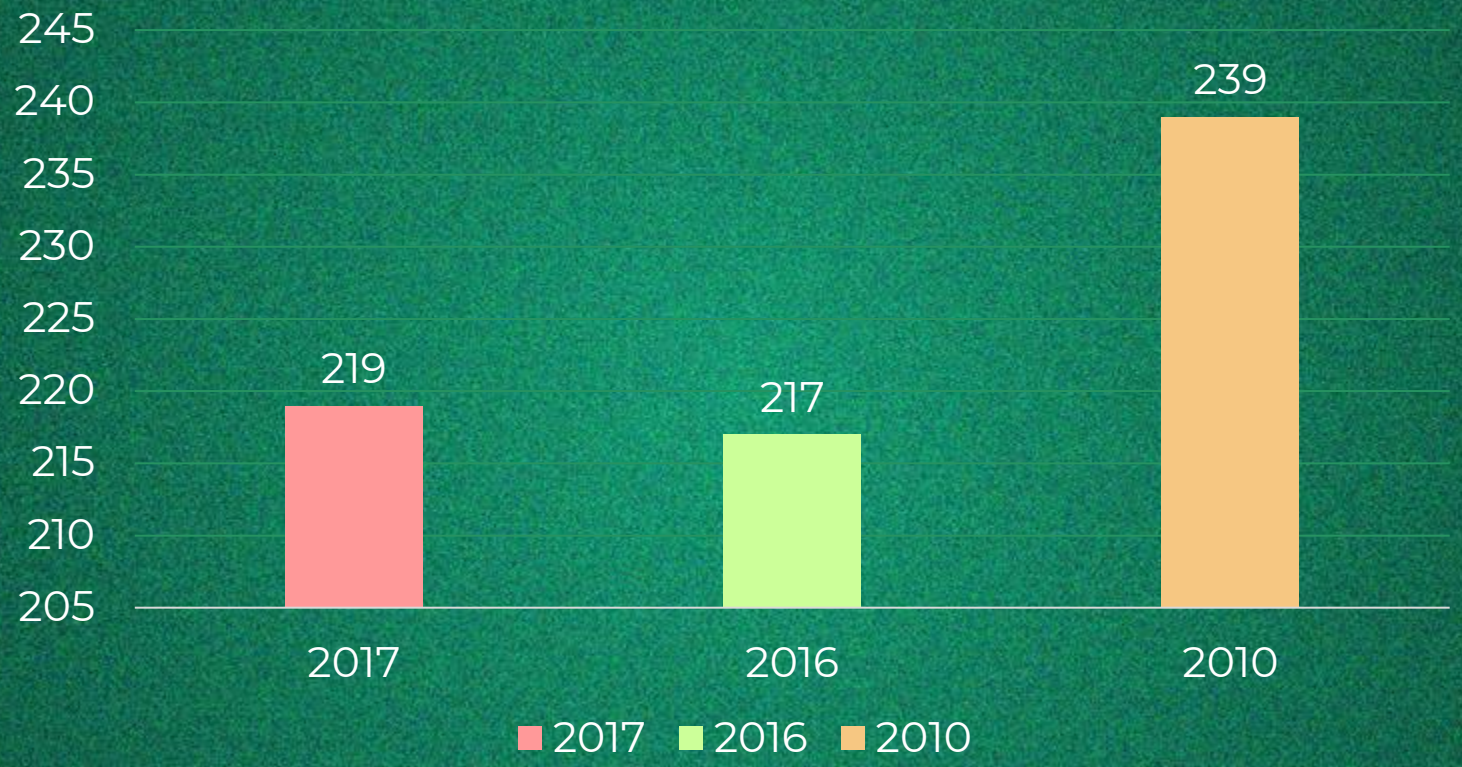
03 **Zika virus**

04 **Yellow Fever**

05 **Filariasis**

INTRODUCTION

The Global and Regional Malaria Burden, in Numbers



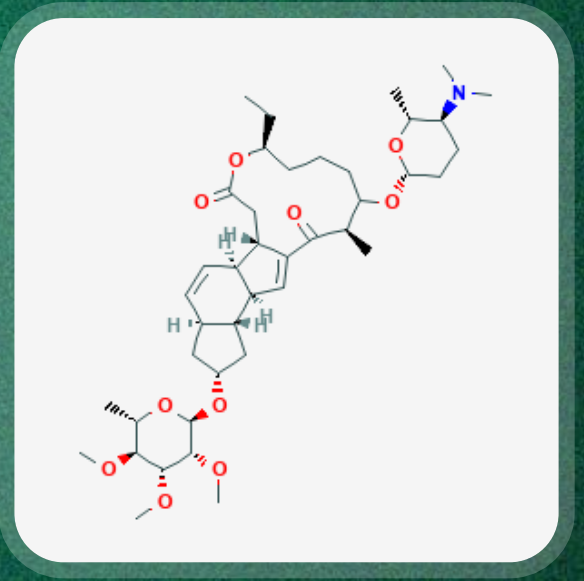
Also, WHO reported that 80% of the global malaria was estimated in 15 countries in sub-Saharan Africa and India in 2017

INTRODUCTION

- **WHO has Considered Mosquito Vector Control** as one of the Important measures to Control Diseases caused by Mosquitoes.
- **The Use of Chemical Insecticides such as** Organophosphate is the Effective Method of Choice.
- **The common Mosquito** Larvicides, Nowadays, include Temephos and Spinosad.
- **Spinosad is a Mixture** of Chemical Compounds in the Spinosyn Family.

It Consists of:

- A unique tetracyclic ring system
- Attached to:
  - **An amino sugar** (D-forosamine)
  - **A neutral sugar** (tri-0-methyl-L-rhamnose)



INTRODUCTION

However, their side effects on human health, environment and development of resistance in mosquitoes against pesticides are causes of concern

About 1 million world-wide deaths and chronic diseases per year were due to the poisoning effect of the pesticide

Production workers, formulators, sprayers, mixers, loaders, and agricultural farm workers are at high risk

INTRODUCTION

That because Pesticides Affect by causing

- **Cancer**
- **Immunosuppression**
- **Congenital Abnormalities**
- **Mental Retardation**
- **Hormonal Disruption**
  where they act as Hormone Antagonist

INTRODUCTION

Recently, concerns increased with respect to public health and environmental security requiring detection of natural products that may be used against insect pests

It is known that larvicides play a vital role in controlling mosquitoes in their breeding sites as mosquitoes breed in water, and thus, it is easy to deal with them in this habitat

INTRODUCTION

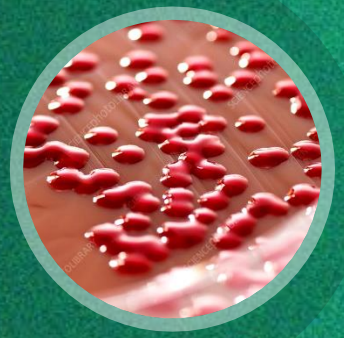
Because of the worsening problems of drug resistance, there has been an urgent need for the discovery of a new chemical class of antimalarial agents

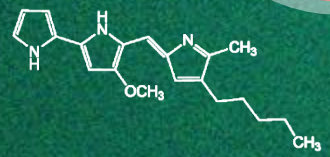
As part of an ongoing natural product research program, microbial extracts have been screened for in vitro antimalarial activity

INTRODUCTION

Prodigiosins

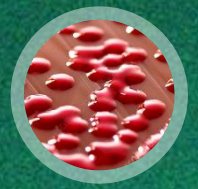
1





Medicinal Plants

2



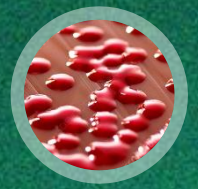
Prodigiosins

Prodigiosins constitute a class of natural products isolated from bacterial strains such as *Serratia marcescens*.

***S. marcescens*, a G-ve Entero bactericeae** has got its attention because of Tripyrrylmethene, a naturally occurring dark red pigment.

Serratia is commonly found in water, soil and is also associated with plants and animals.

They are opportunistic pathogens, especially in compromised hosts, and may cause sepsis, bacteremia, meningitis, osteomyelitis, endocarditis, infections of the urinary and respiratory tracts.



Prodigiosins

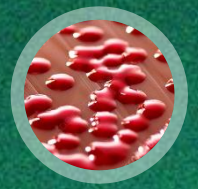
Prodigiosin has been shown to be associated in extracellular vesicles, cell associated or present in intracellular granules.

It belongs to the family Prodigiosinide, which is common to all the members of this family such as:

Cyclo
Prodigiosin

Metacyclo
Prodigiosin

Dipyrrolildipirromethan
Prodigiosin



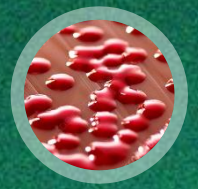
Prodigiosins

Prodigiosin, a bioactive secondary metabolite is characterized by a common pyrrolyl pyrromethane skeleton.

In the PDG molecule (C20H25N30) and a molecular weight of 323.44 Da, the pyrrolyldipyrromethene structure (linear tripyrrole) is composed of two of the rings linked to each other and the third is attached via a methane bridge.

PDG and undecylprodigiosin belong to linear tripyrroles while metacyclo-prodigiosin, prodigiosin R1, and streptorubin belong to cyclic prodigiosins.

The highly conjugated system of seven double bonds is responsible for the intense pigmentation as it forms dark red square pyramidal crystals with a green reflex.

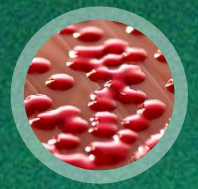


Prodigiosins

Prodigiosin revealed a broad range of inhibitory activities against many bacterial, fungal, and protozoan species

It has antibacterial activity due to Its ability to pass through the outer membrane and inhibiting target enzymes such as DNA gyrase and topoisomerase IV, which inhibited the cell growth.

Prodigiosins are strong therapeutic molecules especially for their immuno-suppressive properties and anticancer properties.



Prodigiosins

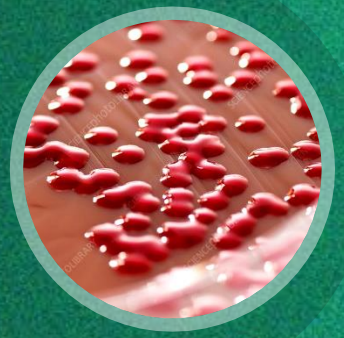
Four possible mechanisms are suggested attributed to prodigiosins as pH modulators, cell cycle inhibitors, DNA cleavage agents and mitogen activated protein kinase regulators.

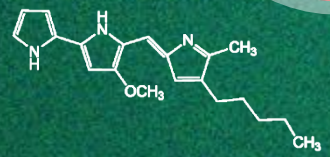
These molecules when combined with some other anticancer agents can greatly help in fighting cancer.

INTRODUCTION

Prodigiosins

1





Medicinal Plants

2

Medicinal Plants

Additionally, during the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world.

Plants generally produce many secondary metabolites which were constituted an important source of many pharmaceutical drugs.

Cupressaceae is a conifer family, the cypress family, with worldwide distribution.

A decoction of the cones and leaves of *Cupressus sempervirens* was used for treating hemorrhoids, varicose veins and venous circulation disorders, bronchitis, head colds and coughs externally.

Medicinal Plants

Internally, the dried cones and young branches are used as deodorant and diuretic. anti-parasitic, antipyretic, antirheumatic, antiseptic, balsamic, vasoconstrictive, and antifungal.

Thuja orientalis has been exhibiting extensive biological activities including anticancer, antiepileptic, antiinflammatory, antibacterial, antifungal activities, hair growth-promoting, antiviral, antiallergic, antioxidant and molluscicidal.

Previously, it was reported that there are volatile oils have more than 50% larvicidal effect after 24 hours.

Medicinal Plants

Essential Oils

Essential oils are called volatile or ethereal oils, due to their evaporation when exposed to heat in contrast to fixed oils.

They are hydrophobic, and are soluble in alcohol, non-polar or weakly polar solvents.

Their density is usually lower than water, and they are oxidizable by light, heat, and air due to their molecular structures.

Essential oils are highly complex mixtures of volatile compounds containing about 20 to 60 individual constituents, and some may contain more than 100 different components such as:

Lemon **Jasmine** **Cinnamon**

Medicinal Plants

Essential Oils

The major volatile constituents are classified into two main categories:

- **Terpenoids**
- **Phenylpropanoids**
- **Hydrocarbons**
- **Oxygenated Compounds**

Medicinal Plants

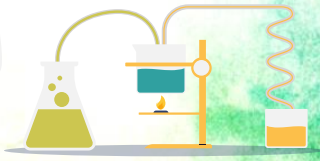
Essential Oils

Pharmacological and medicinal importance of Essential oils:

- **Perfumes**
- **Cosmetics**
- **Phytotherapy**
- **Aromatherapy**
- **Insecticides**
- **Herpes Simplex Virus**
- **Coronary Heart Diseases**
- **Leukemia**

Aim of the Work

CHAPTER TWO



Novel Formula

as Mosquito Larvicides

PLAN OF THE WORK

PLAN OF THE WORK

Plan 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Plan 02

Preparation and characterization of four essential oils from fresh leaves of *Thuja orientalis* (TOL).

Plan 03

Preparation and characterization of four extracts from dried leaves of studied plants

Plan 04

Maintaining the mosquito by rearing the culture of Cx pipiens

PLAN OF THE WORK

Plan 05 Dose response bioassay separately of all preparations and dry extract.

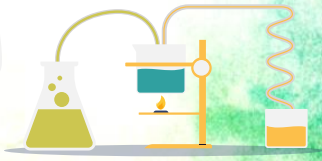
Plan 06 Investigation for the synergistic effect of PDG with each E.O, and dry extract individually as mosquito larvicidal potential.

Plan 07 Investigating the mode of action of PDG, E.O and dry extract for mosquito larvicidal potentially.

Plan 08 Probit analysis for calculating the lethal concentration of PDG, E.O and dry E.

Materials and Methods

CHAPTER THREE



STUDY SETTING

01 STUDY

Biotechnology in the Institute of Graduate Studies and Researches
Alexandria University

02 STUDY

Pharmacognosy at Faculty of Pharmacy
Alexandria University

03 STUDY

Vector Control and Pesticide Risks, Tropical Health Department, High Institute of Public Health
Alexandria University

04 STUDY

City of Scientific Research and Technological Applications

05 STUDY

Drug Research Center of the Faculty of Pharmacy
Pharos University in Alexandria

Materials

Bacteria Strain
***Serratia Marcescens* Strain**

***Serratia Marcescens* Strain** was obtained from other colleagues in the Biotechnology Department - IGSR

Animals and insects
Larvae and Pupae

Larvae and pupae of Culex pipiens were purchased from the Institute of Medical Insects in El-Dokki, Cairo-Egypt.

Pigeons were purchased from the market and used as a source of blood meal for the adult female insects.

Plant Materials

Plant materials Fresh leaves and cones of ***Thuja orientalis*** were collected from Anotoniadis Botanical Garden in Alexandria.

Silica Gel and PDG Standard

Silica Gel and PDG standard Were obtained from Sigma Aldrich (USA)

Materials

01 Solvent systems for TLC

02 Adsorbents

03 Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

Hood

Instrumentation and special apparatus

Materials

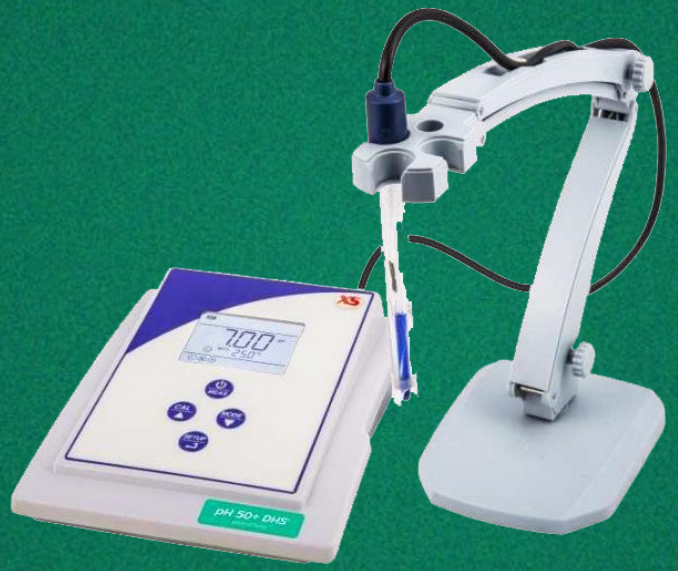
03 Instrumentation and special apparatus

Shaker incubator

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus



pH meter

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

Autoclave

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

Digital Balance

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

Fermenter

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

GC-MS

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

Rotary Evaporator

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

UV-Spectrophotometer

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

FT-IR

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus

HPLC

Instrumentation and special apparatus

Materials

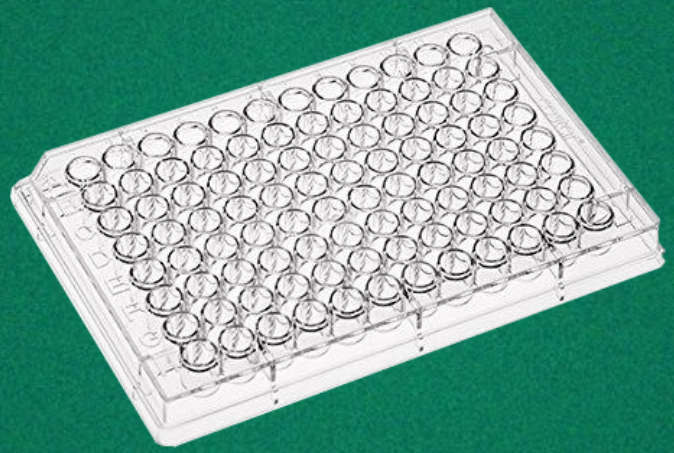
03 Instrumentation and special apparatus

Eppendorf Cooling Centrifuge

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus



Microplates

Instrumentation and special apparatus

Materials

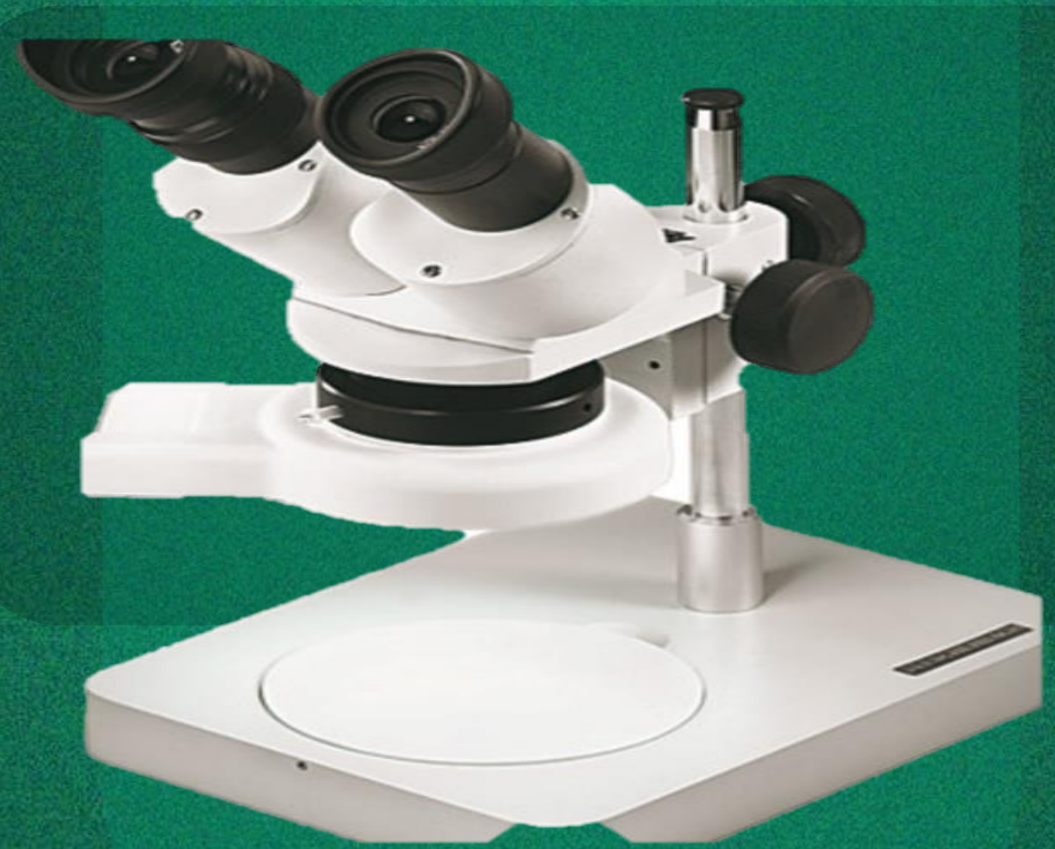
03 Instrumentation and special apparatus

Glass Wares, Automatic Pipettes

Instrumentation and special apparatus

Materials

03 Instrumentation and special apparatus



Stereomicroscope

Instrumentation and special apparatus

Methods

Method
01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

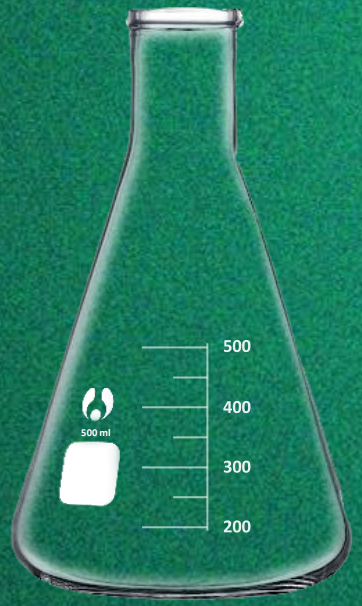
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

PY Media



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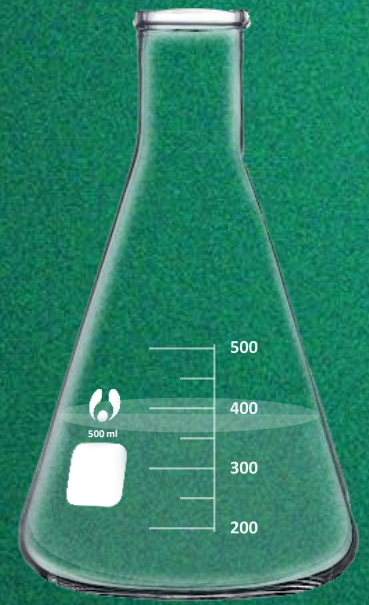

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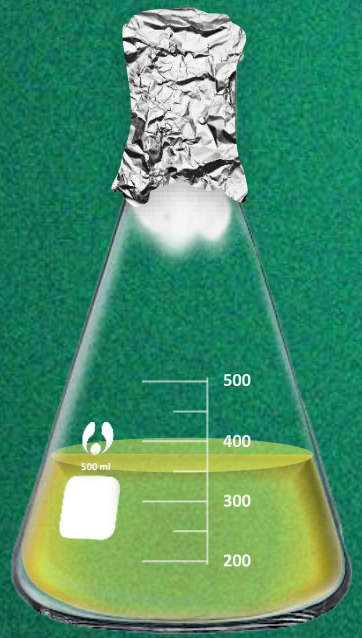

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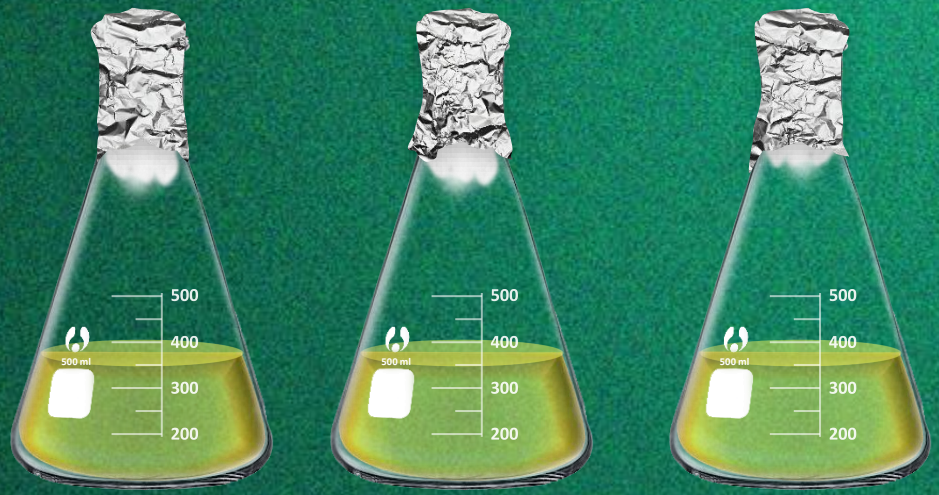

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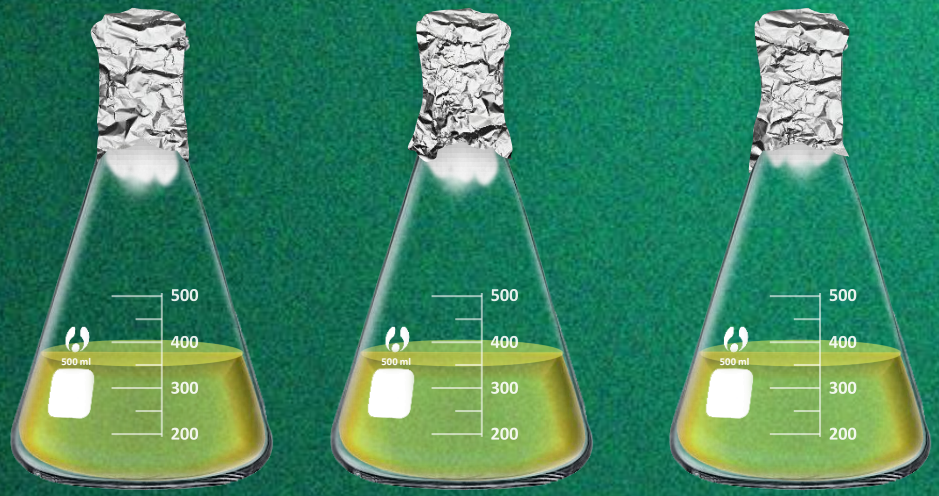

Methods

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Peanut Media

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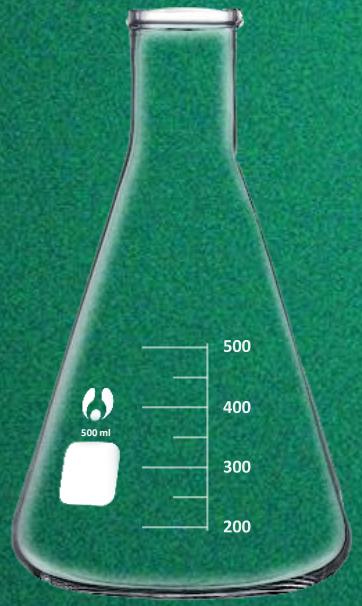

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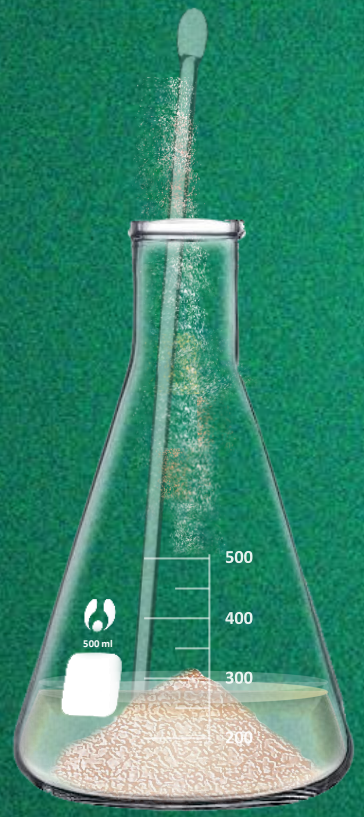

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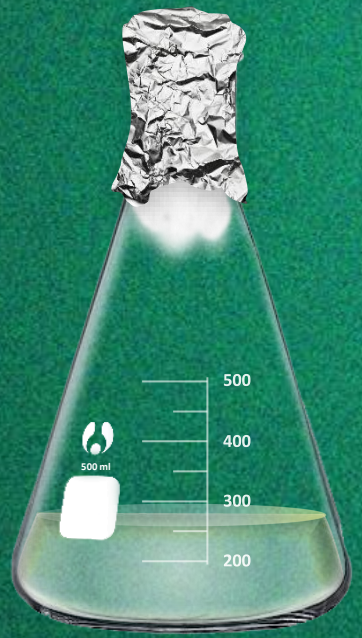

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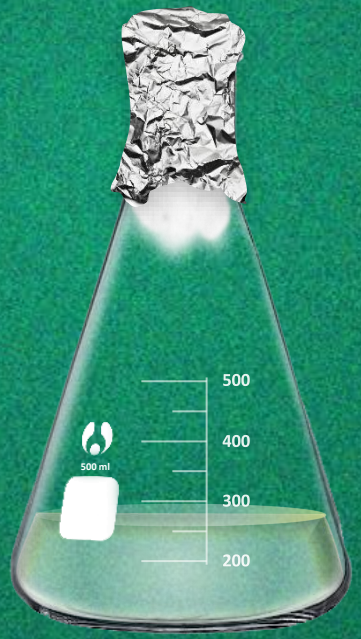

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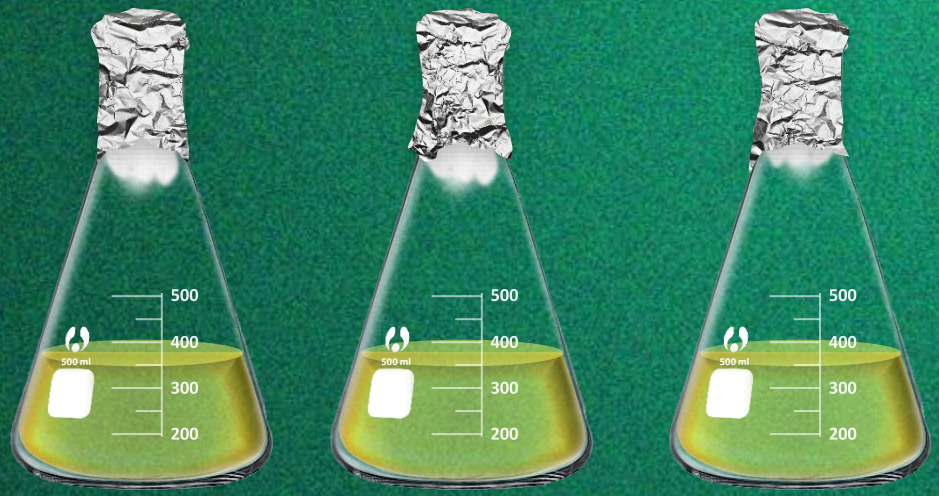
Inoculation of PY Media



50 ml | 50 ml | 50 ml

1 ml
Crude Prodigiosin in Ethanol

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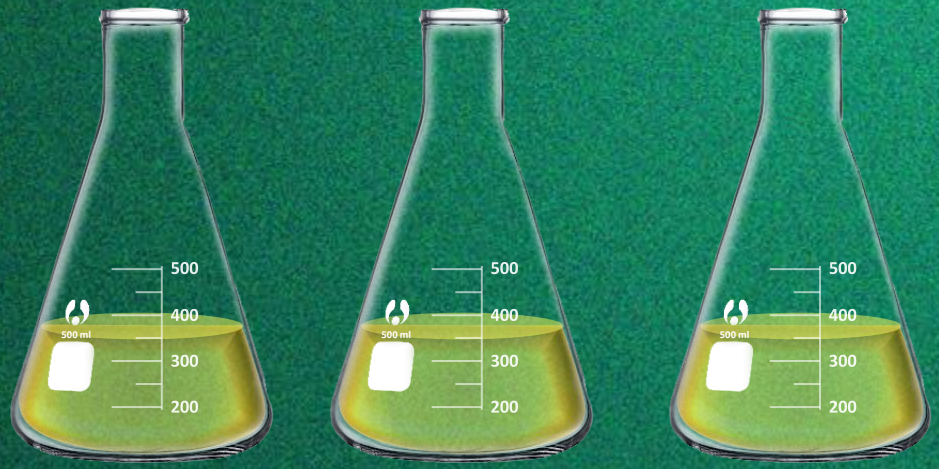

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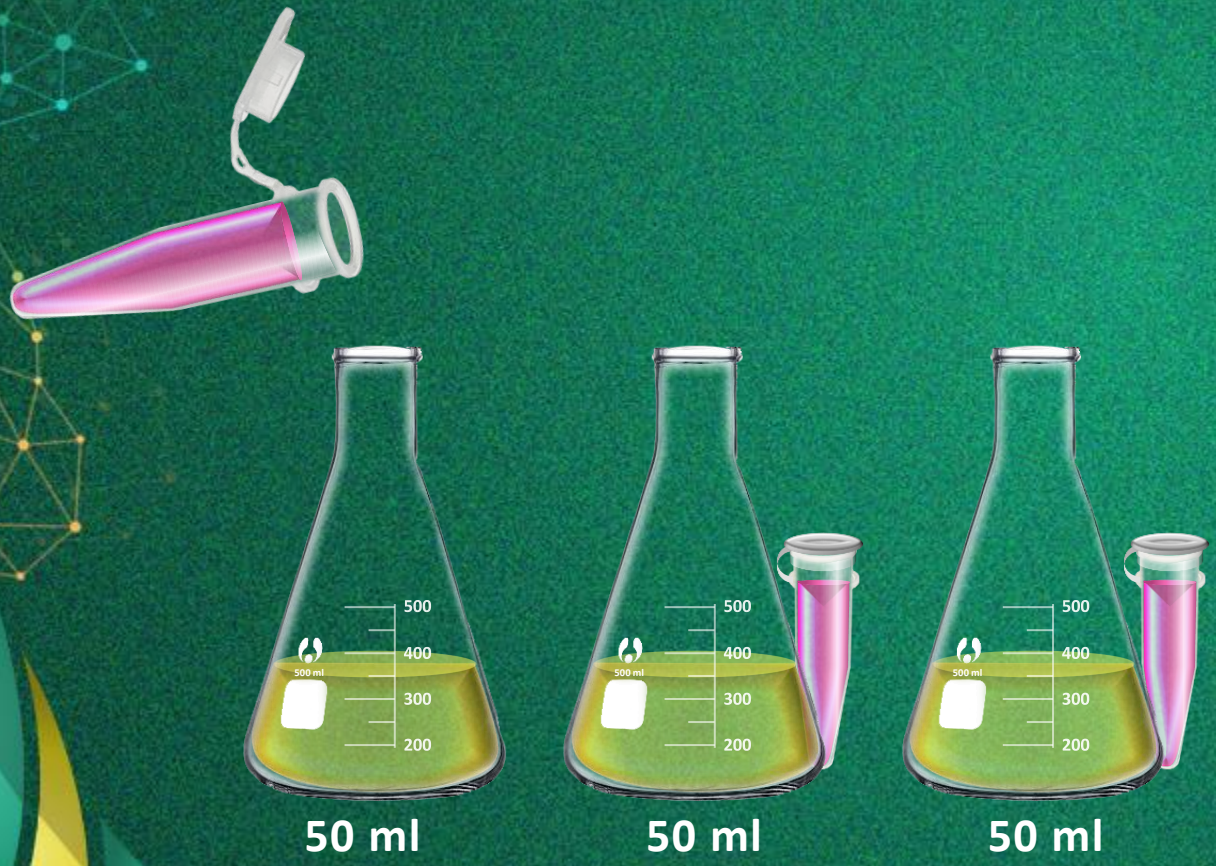

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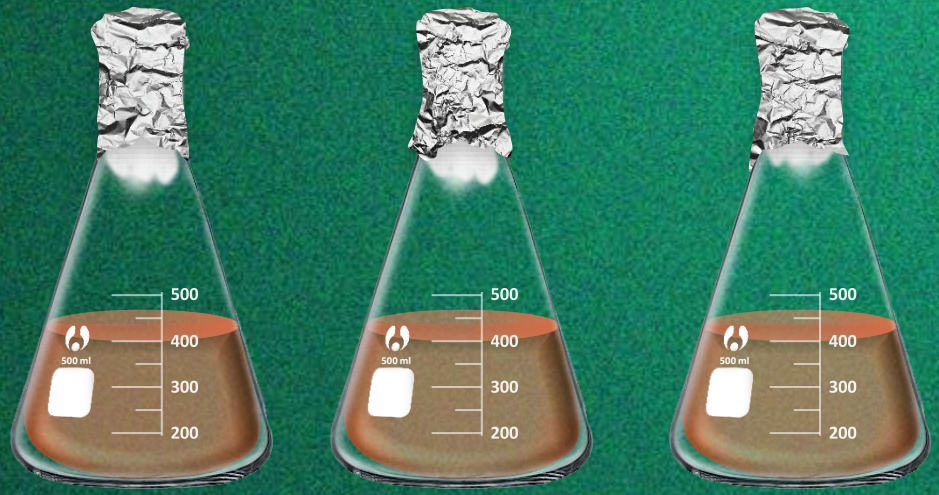

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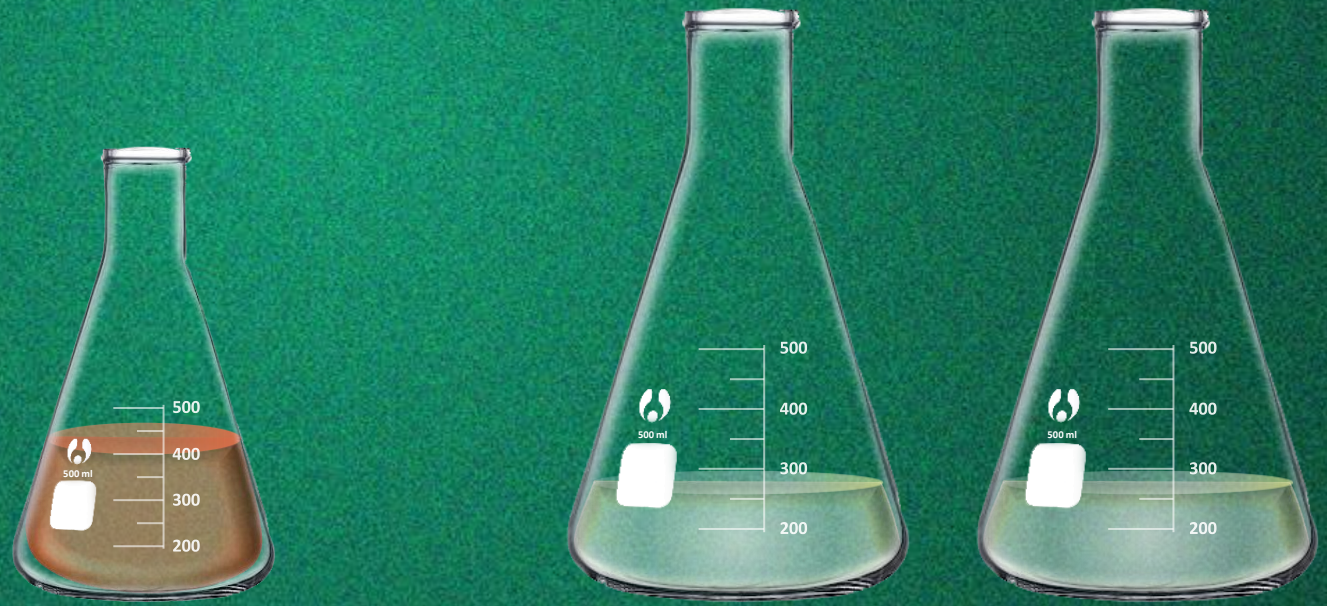
Growth and activation of *S. marcescens*

Inoculation of Peanut Media



Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Inoculation of Peanut Media

25 ML

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Inoculation of Peanut Media

25 ML

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Inoculation of Peanut Media

25 ML

Methods

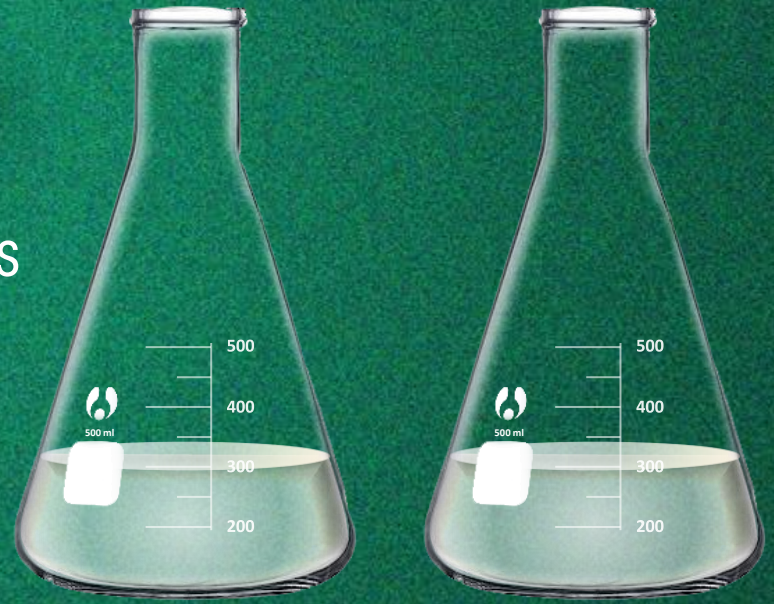
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Inoculation of Peanut Media

48 Hours



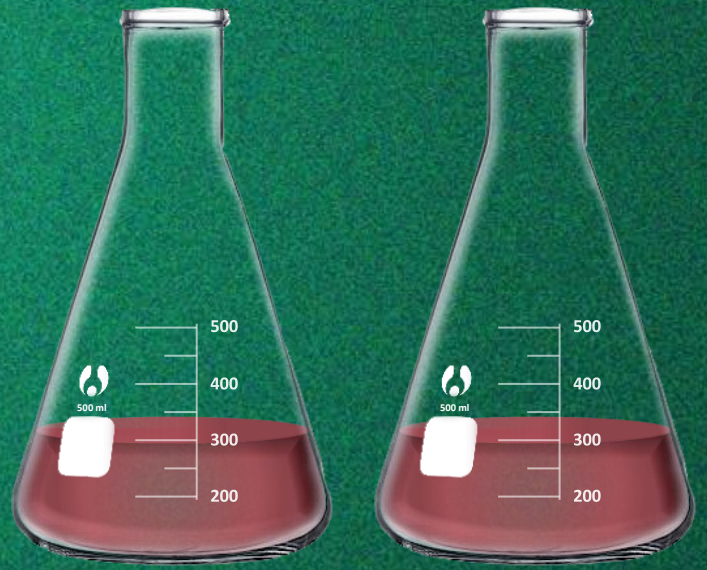
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Inoculation of Peanut Media



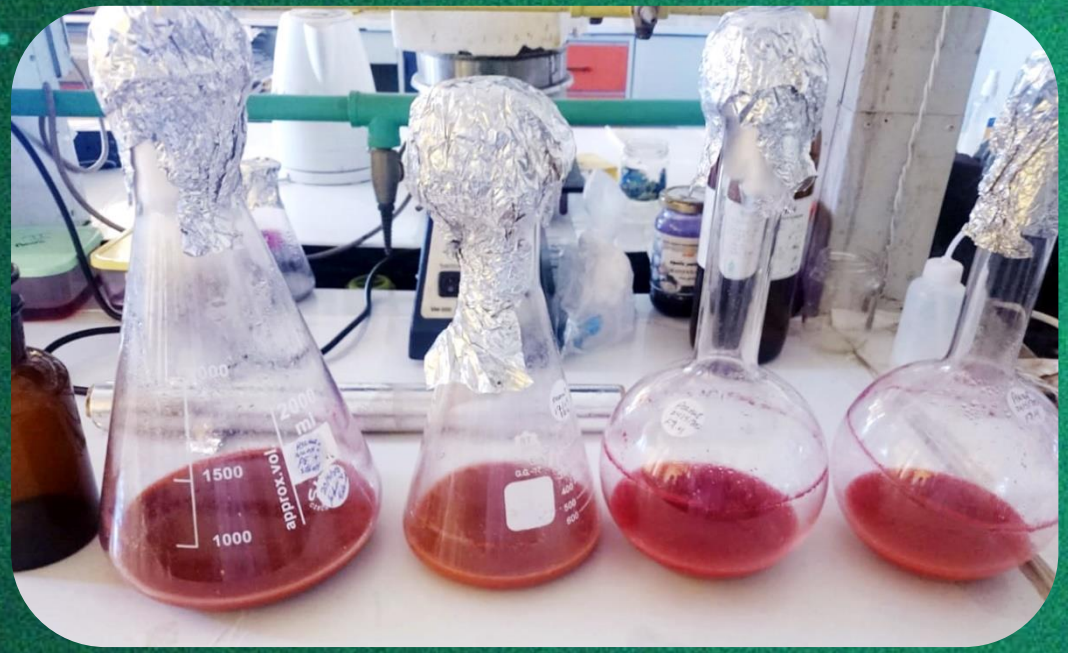
Methods

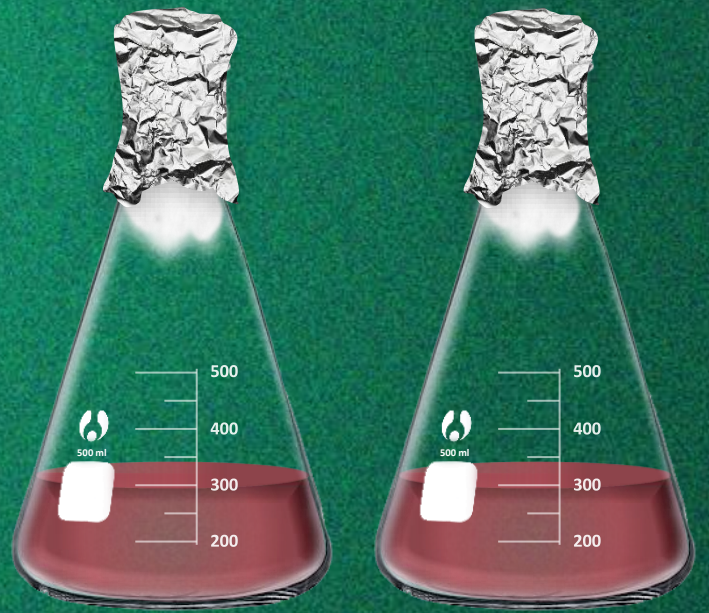
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Red pigment





Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Growth and activation of *S. marcescens*

Fermenter

Methods

Method
01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

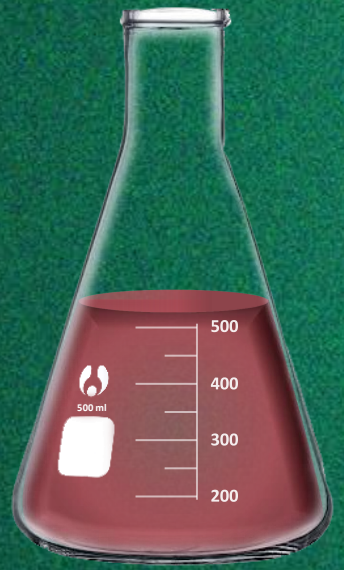
Extraction of Prodigiosin

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



NaOH 100 gm

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

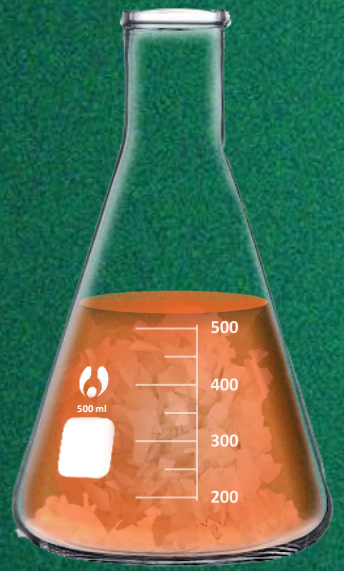
NaOH 100 gm

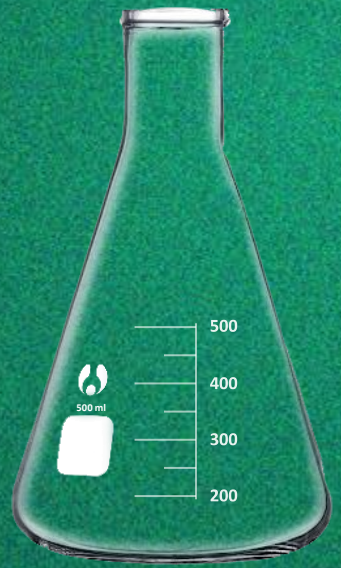
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin





Petroleum Ether

Ethanol

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

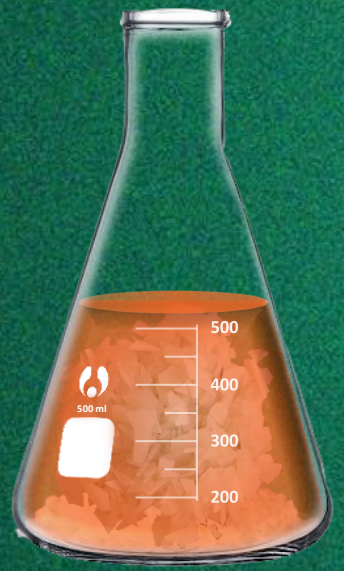
Extraction of Prodigiosin

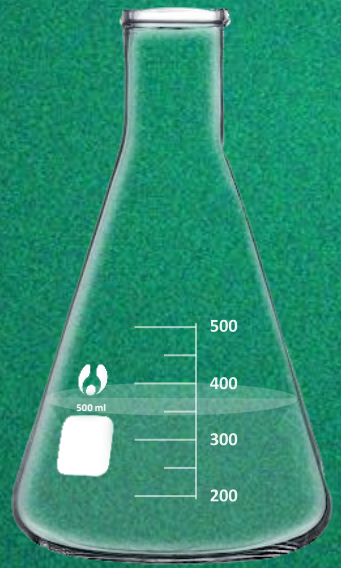
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin





Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

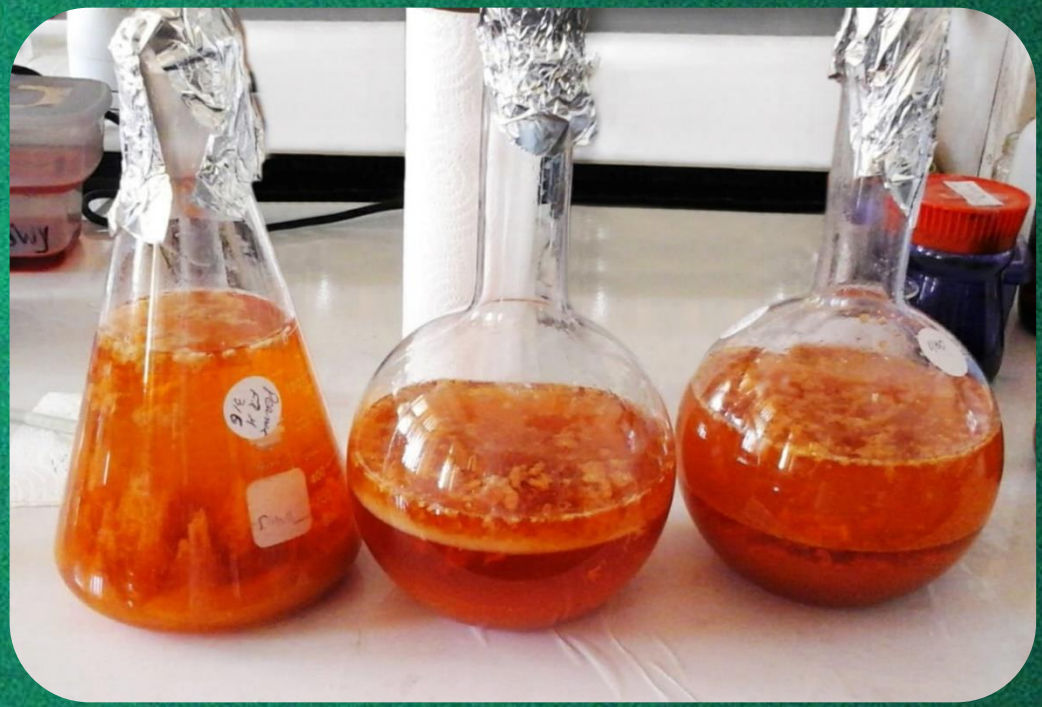
Extraction of Prodigiosin

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



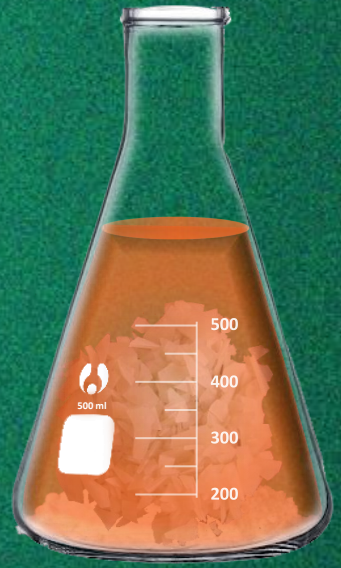
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

48 Hours

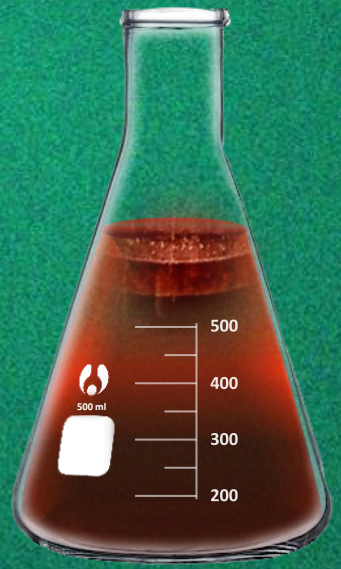


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

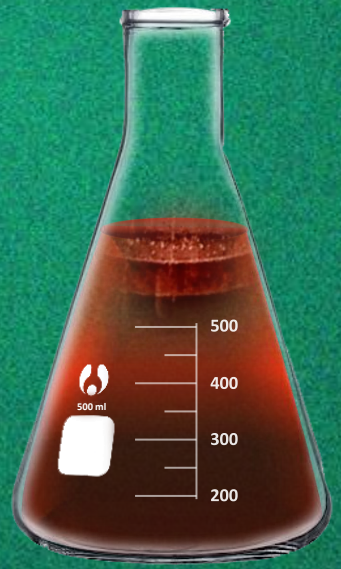


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



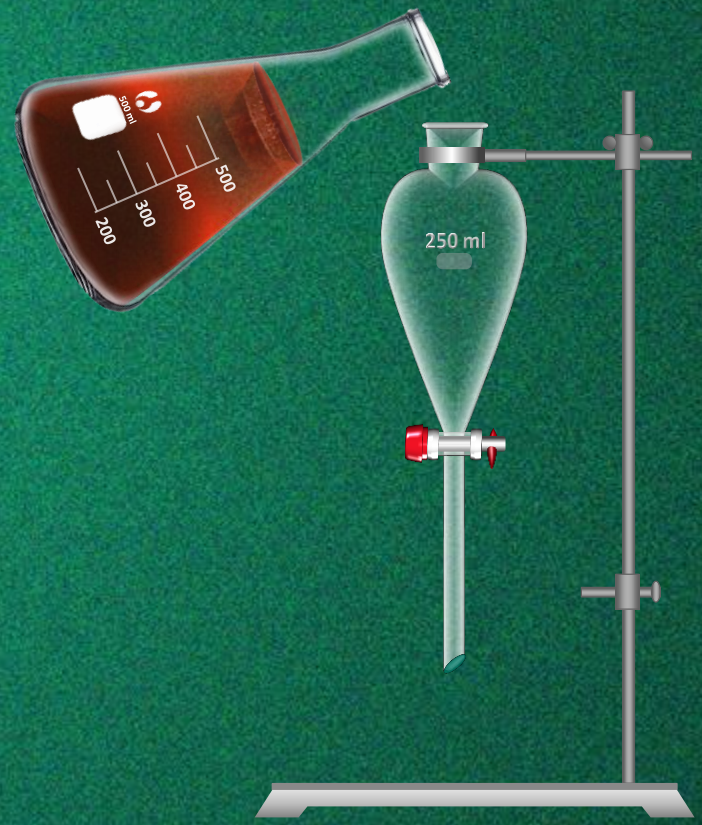
Mode
Close

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

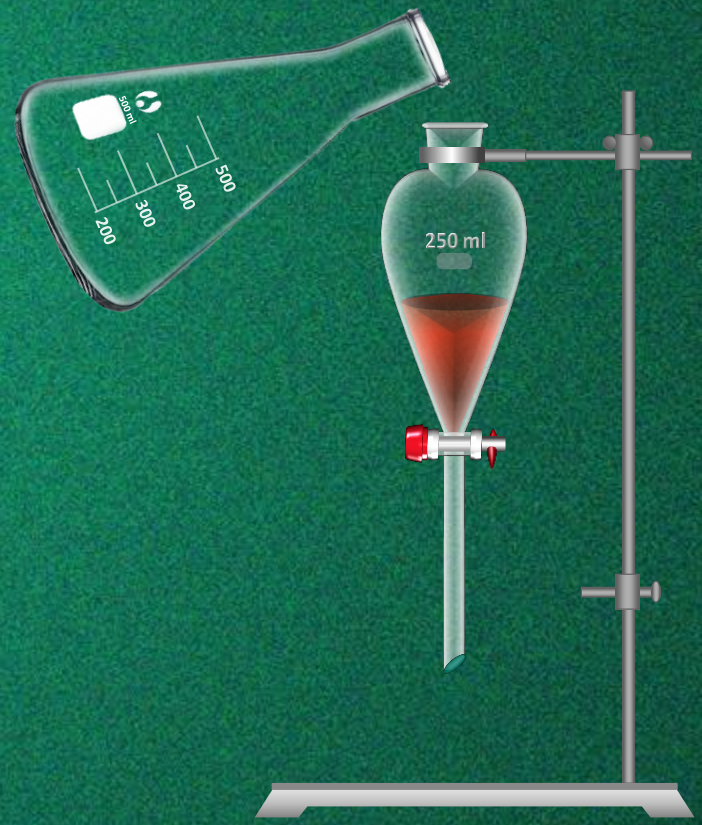


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

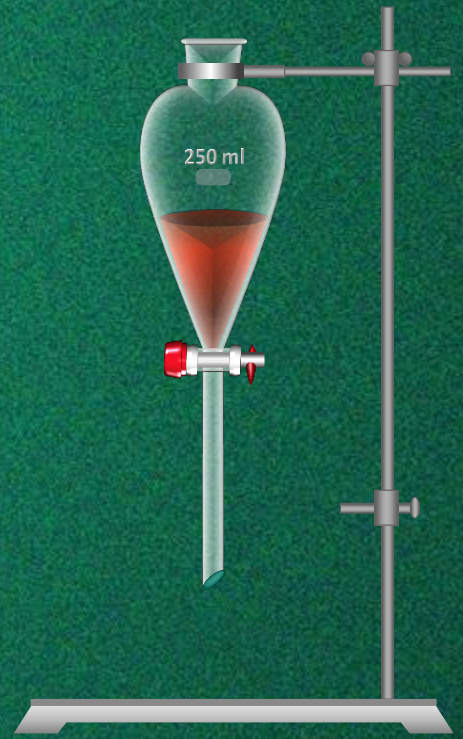


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

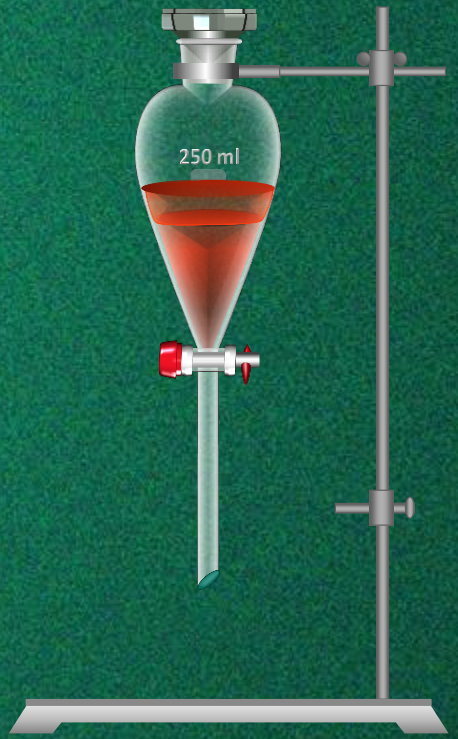
Extraction of Prodigiosin

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

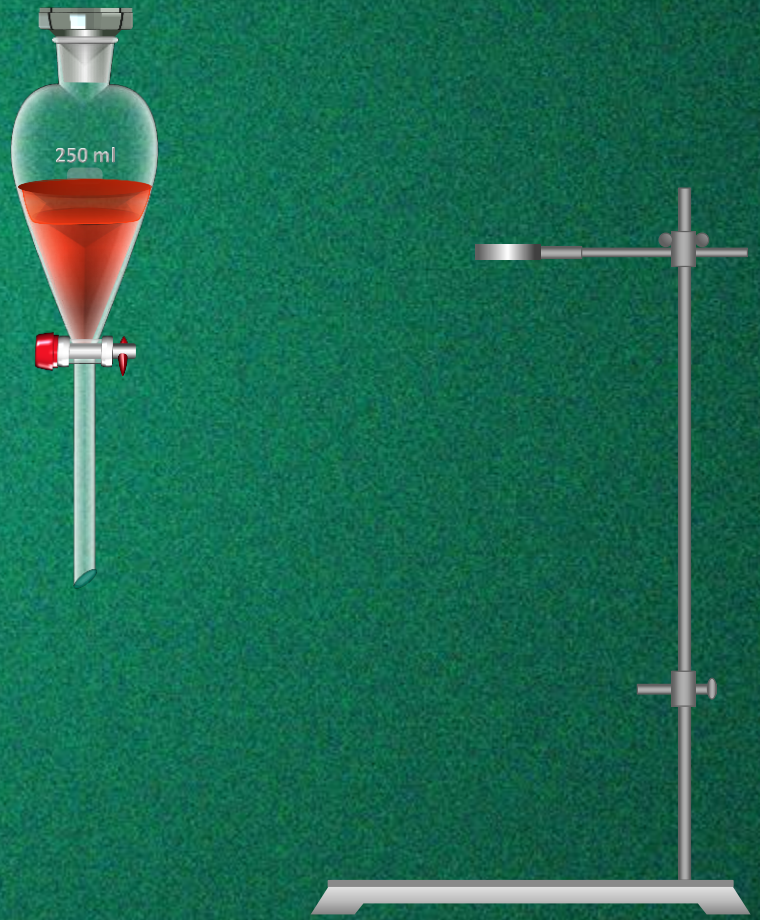


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

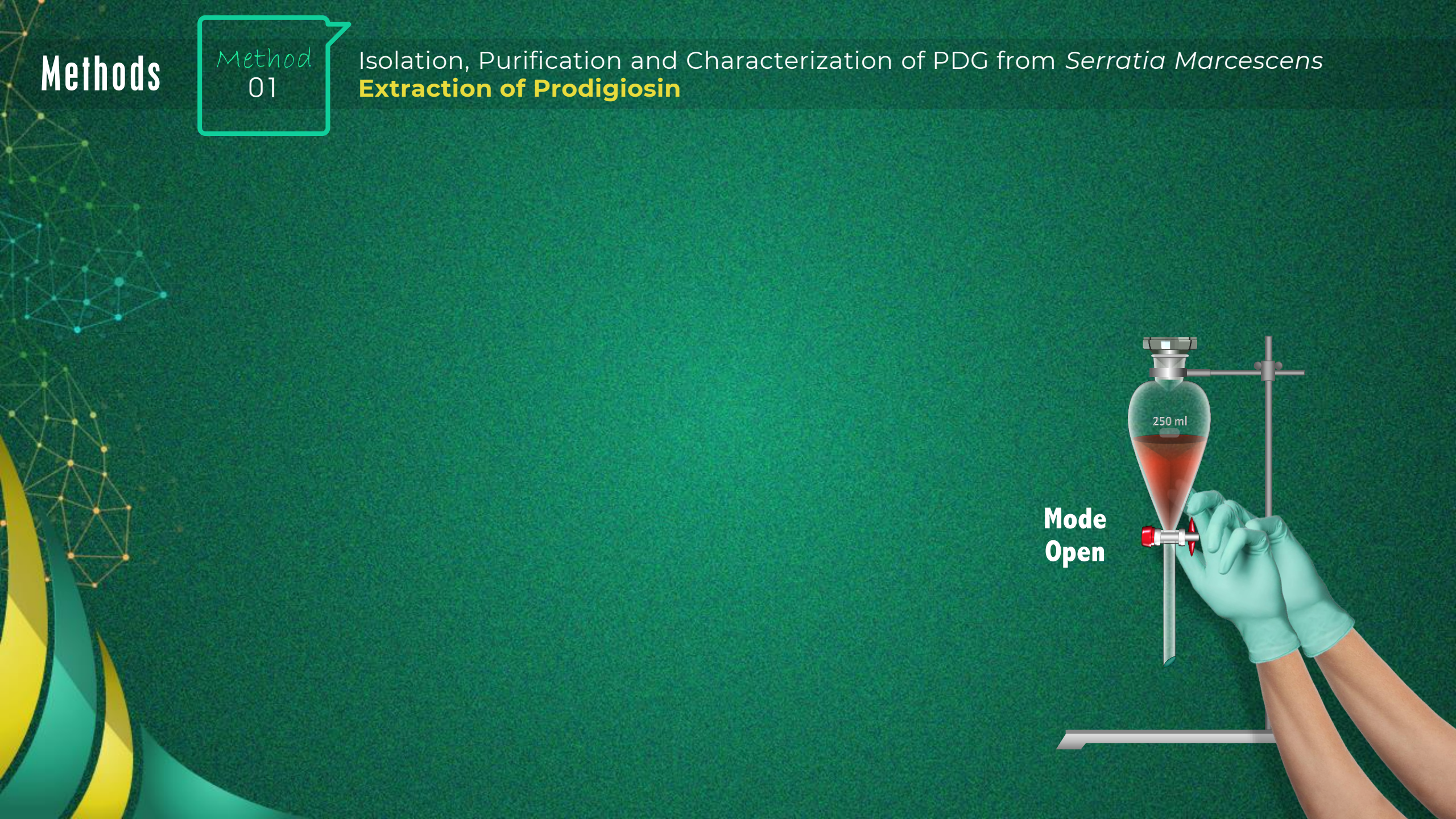


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

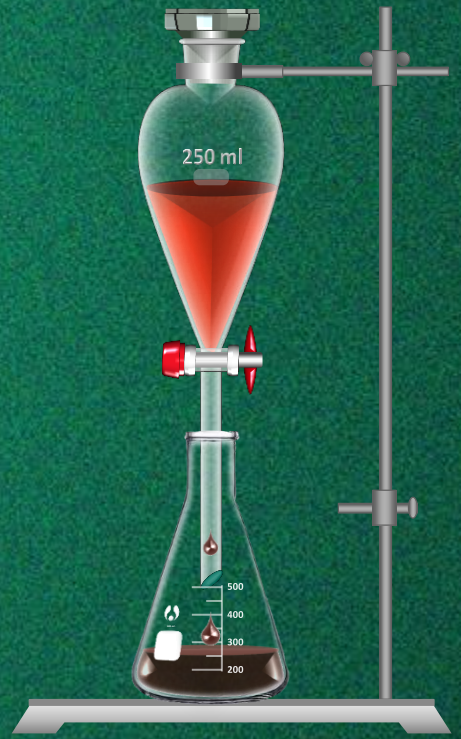


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

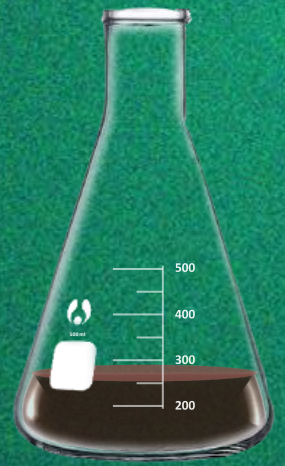


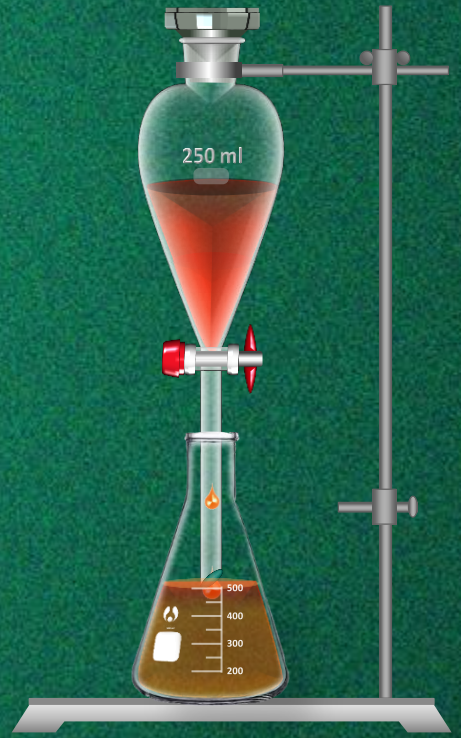
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



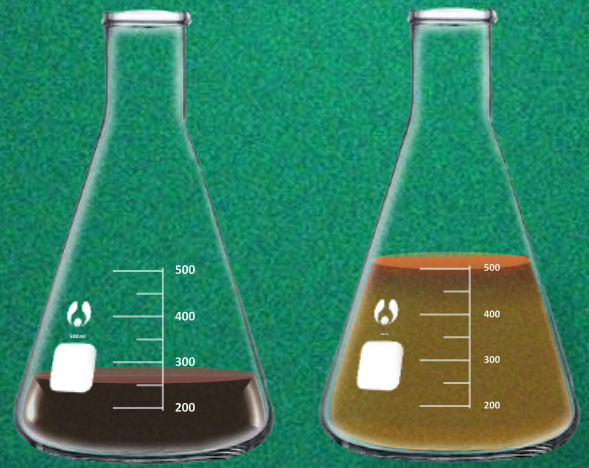


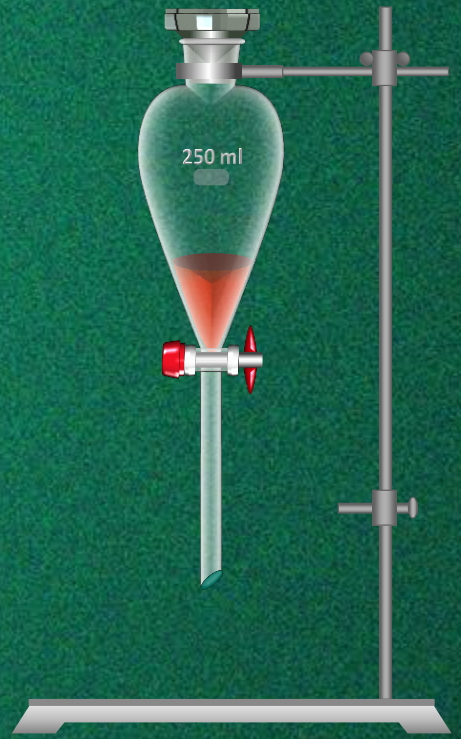
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



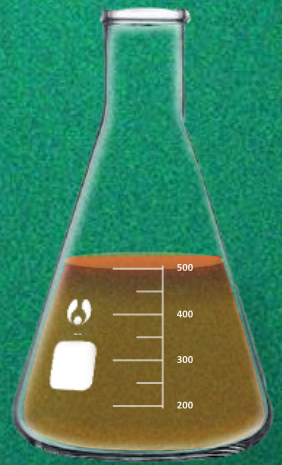


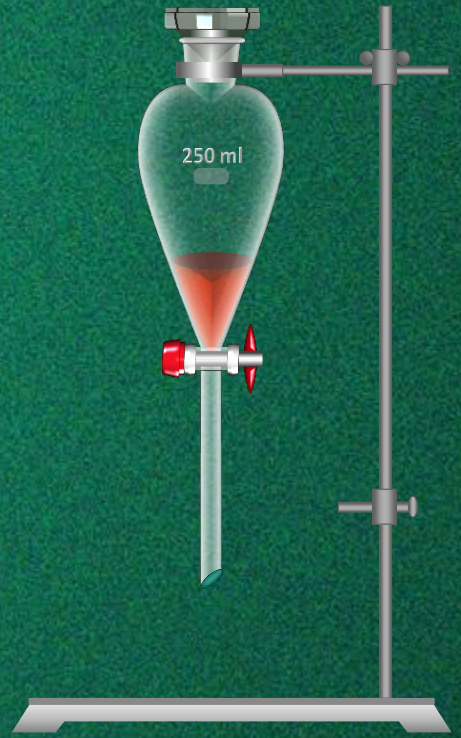
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin



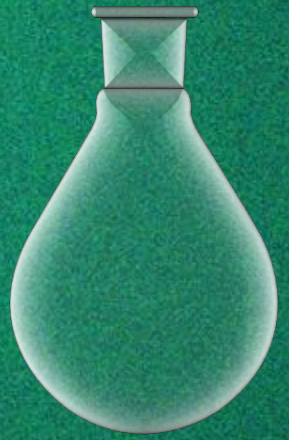


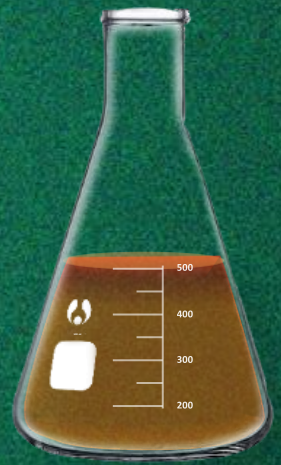
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin





Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

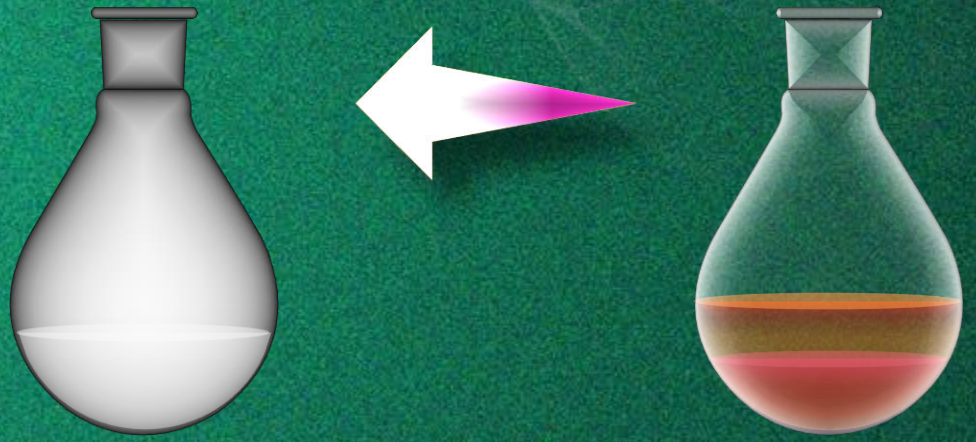
Extraction of Prodigiosin

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

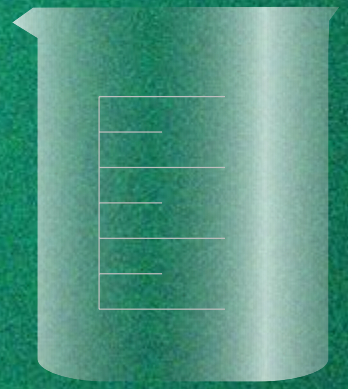


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

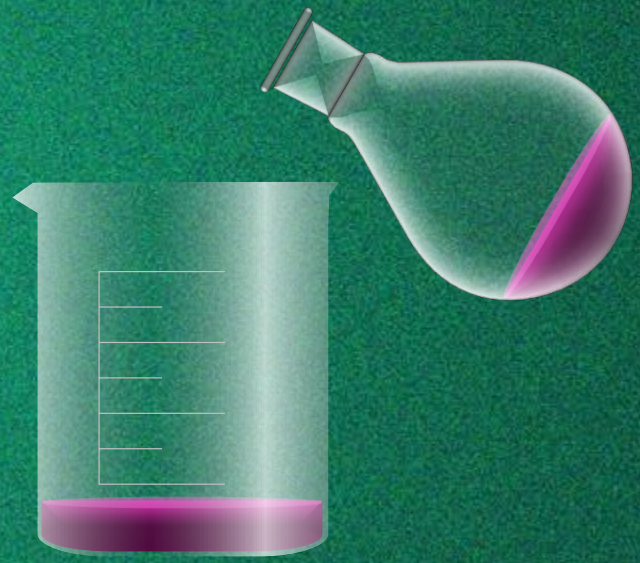


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

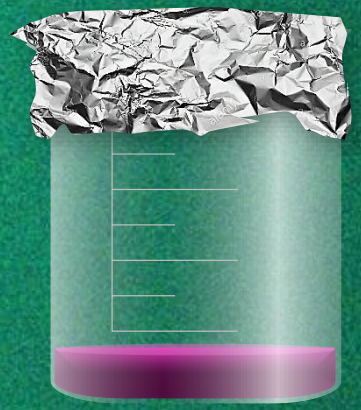


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin

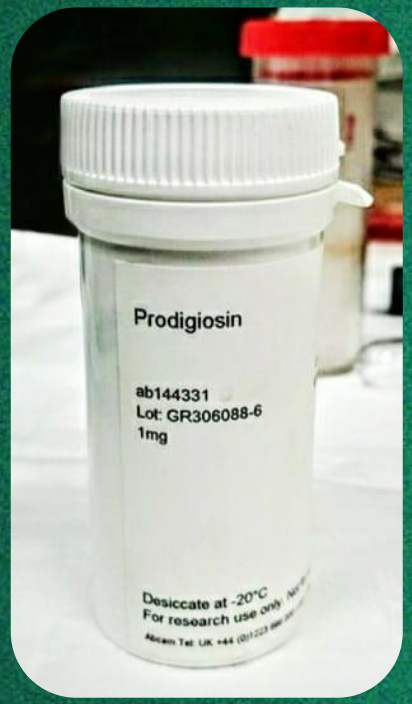


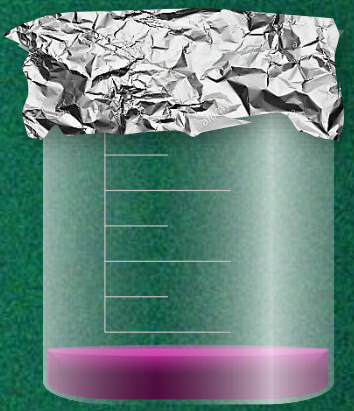
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Extraction of Prodigiosin





Methods

Method
01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

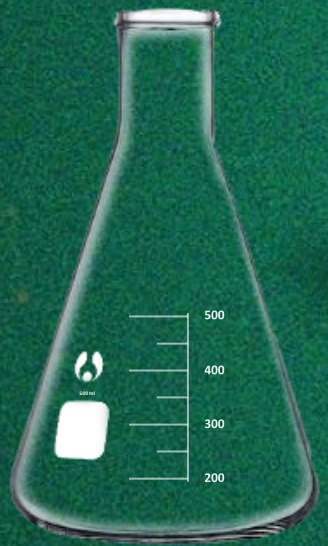
Purification of prodigiosin (Column Chromatography)

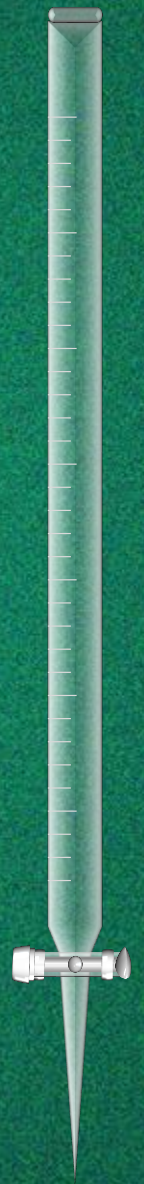
Methods

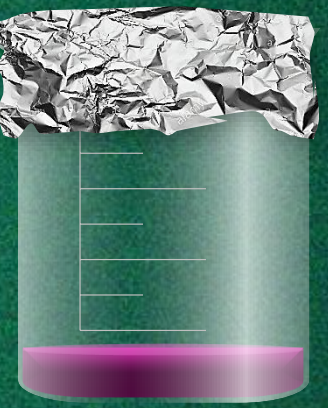
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





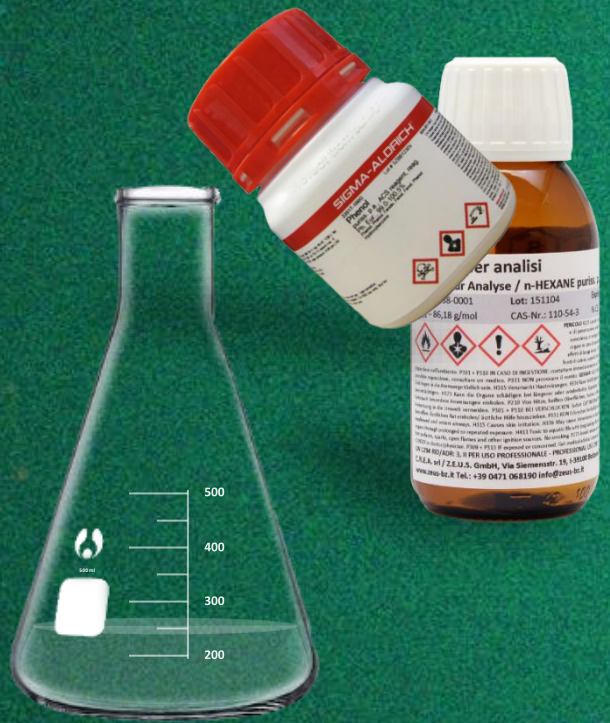


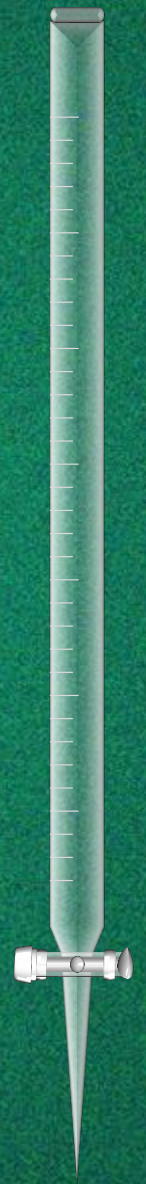
Methods

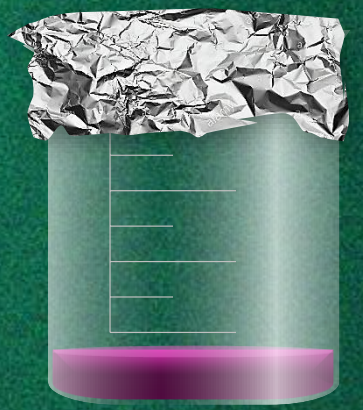
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





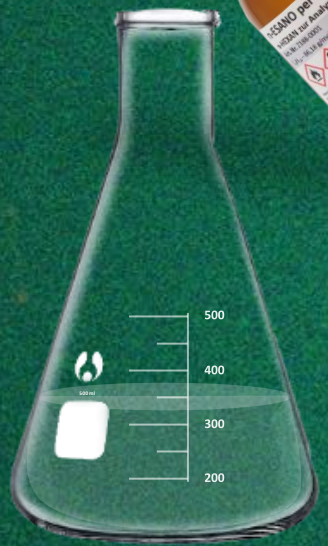


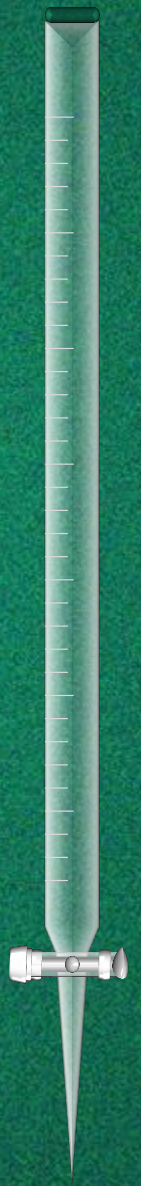
Methods

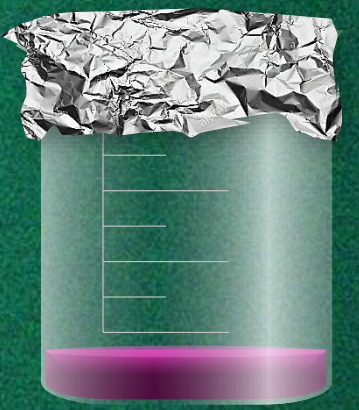
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





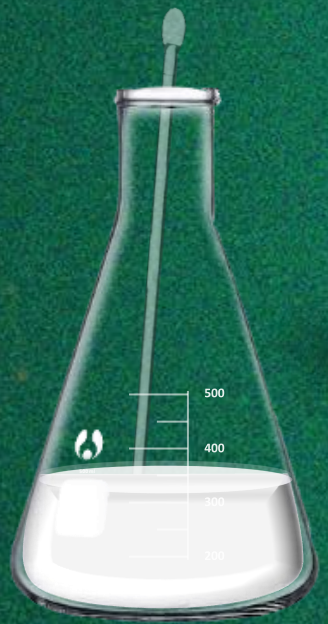


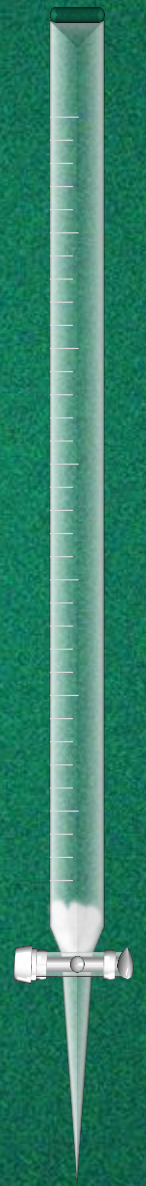
Methods

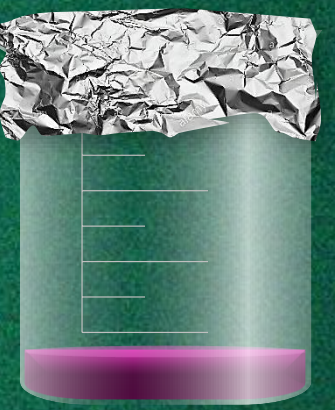
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)







Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

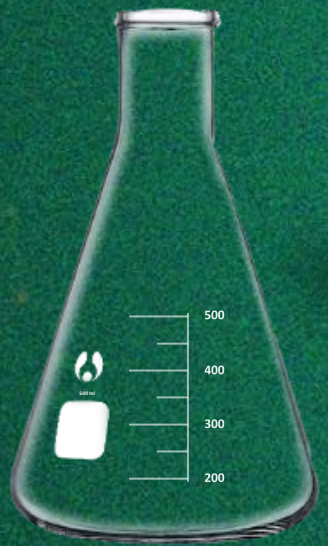
Purification of prodigiosin (Column Chromatography)

Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)

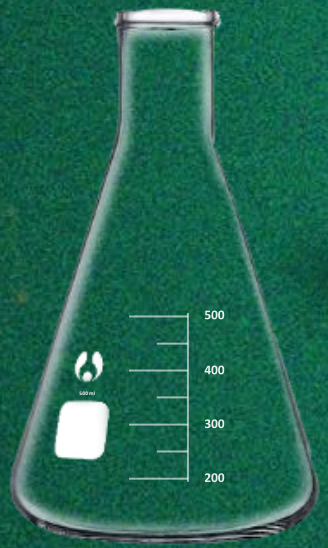


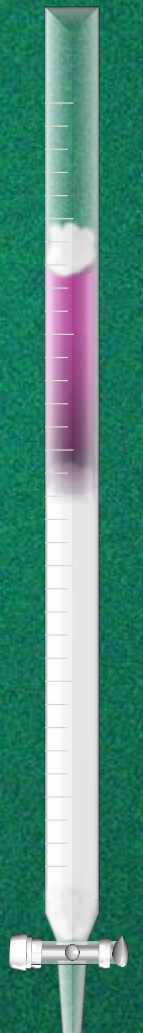
Methods

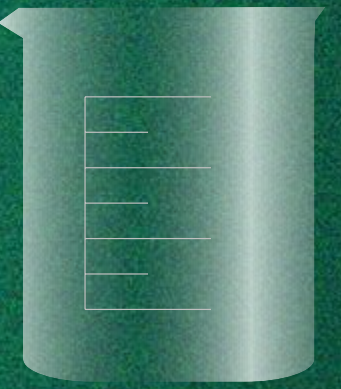
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





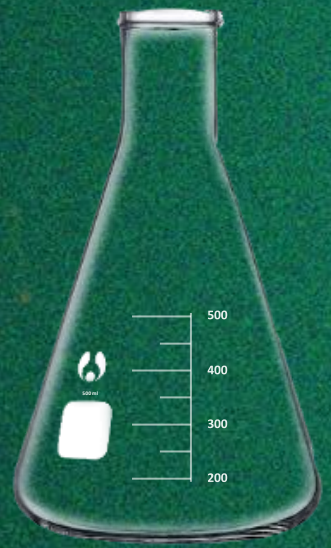


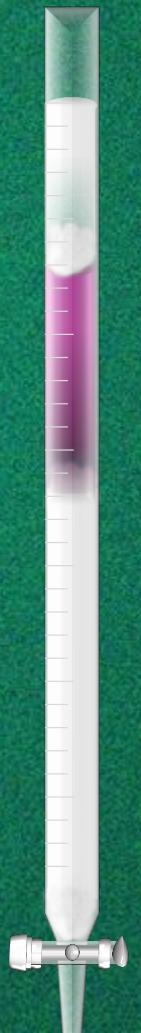
Methods

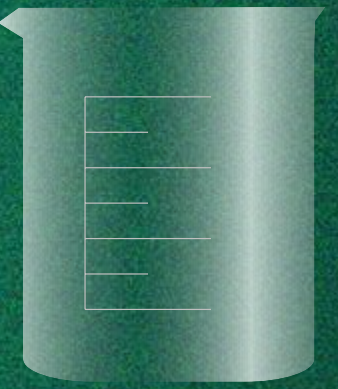
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





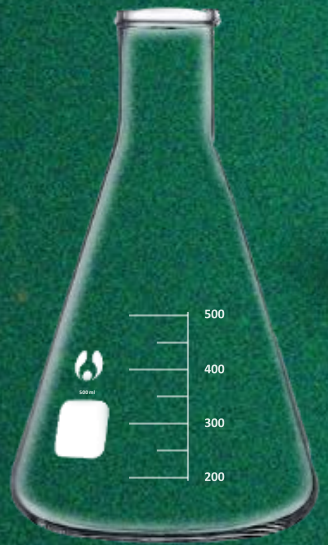


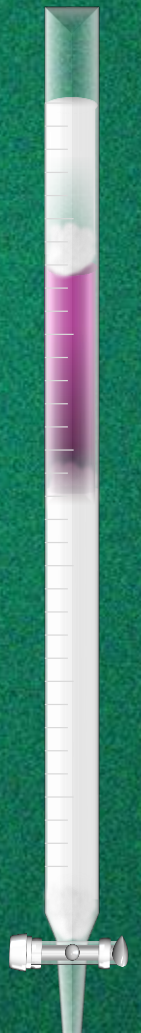
Methods

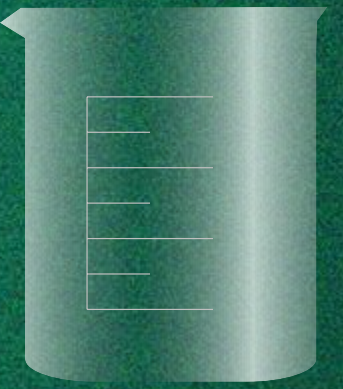
Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





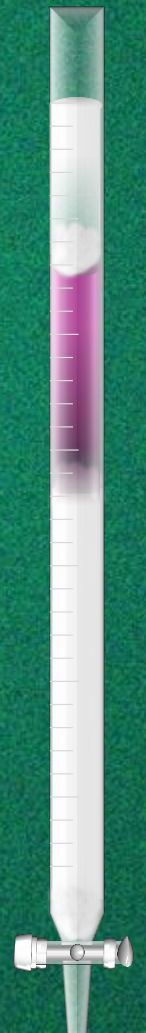


Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)



Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

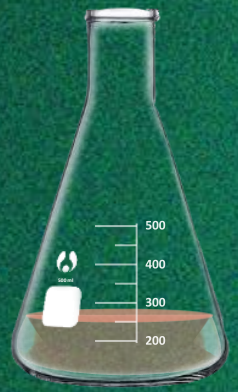
Purification of prodigiosin (Column Chromatography)

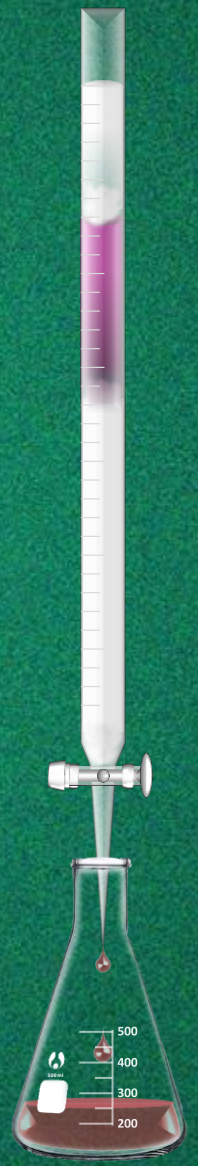
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)



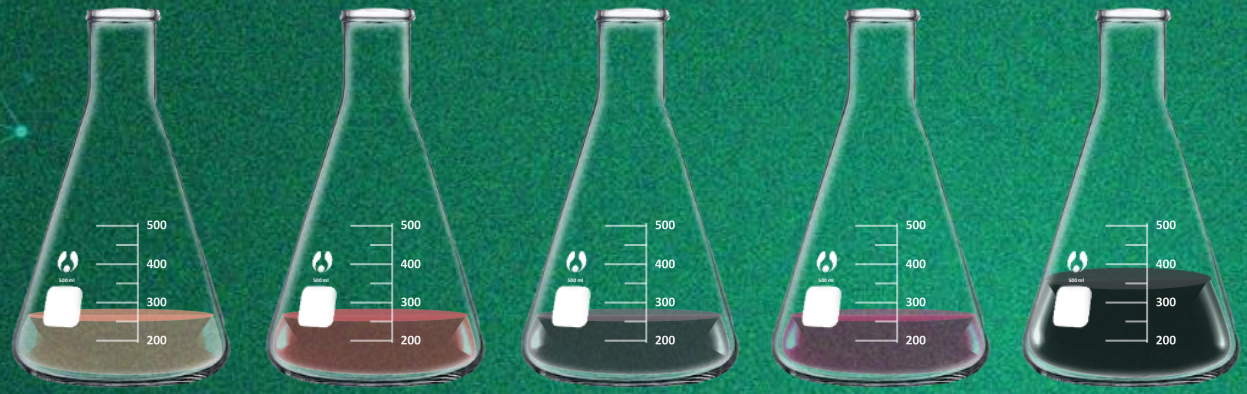


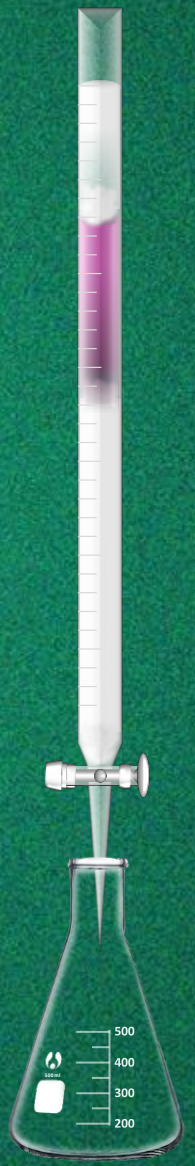
Methods

Method 01

Isolation, Purification and Characterization of PDG from *Serratia Marcescens*

Purification of prodigiosin (Column Chromatography)





Methods

Method
02

Preparation of Four Essential Oils from Fresh Leaves

Methods

Method 02

Preparation of Four Essential Oils from Fresh Leaves

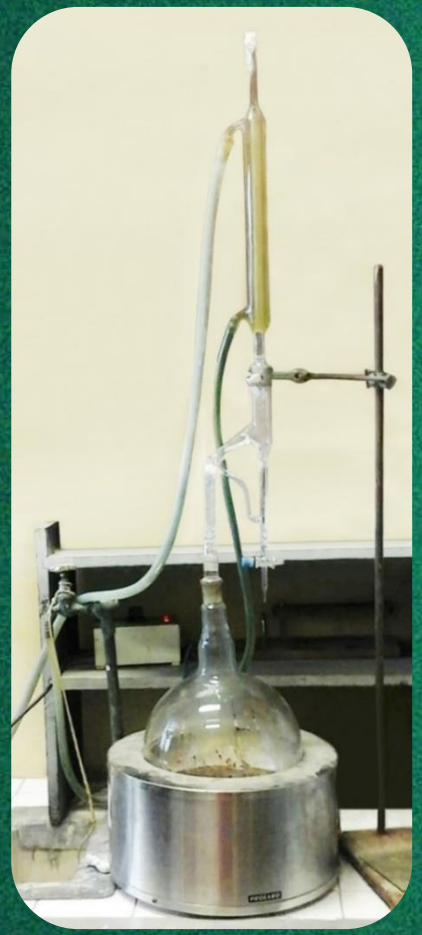
T. orientalis

Leaves

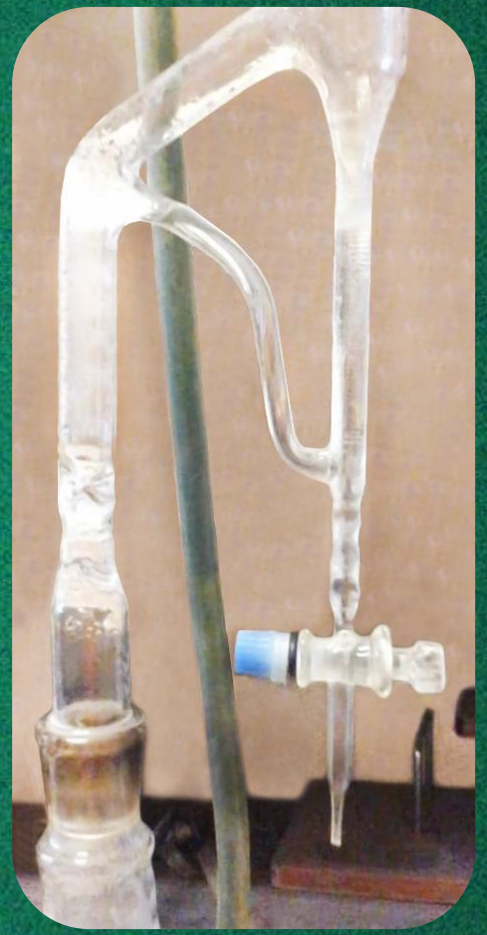
Methods

Method 02

Preparation of Four Essential Oils from Fresh Leaves



Steam Distillation of leaves



Condensation of the oil

Methods

Method
03

Preparation of Four Extracts from Dried Leaves of Studied Plant

Methods

Method 03

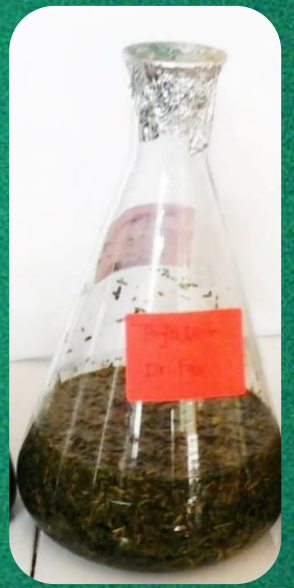
Preparation of Four Extracts from Dried Leaves of Studied Plant

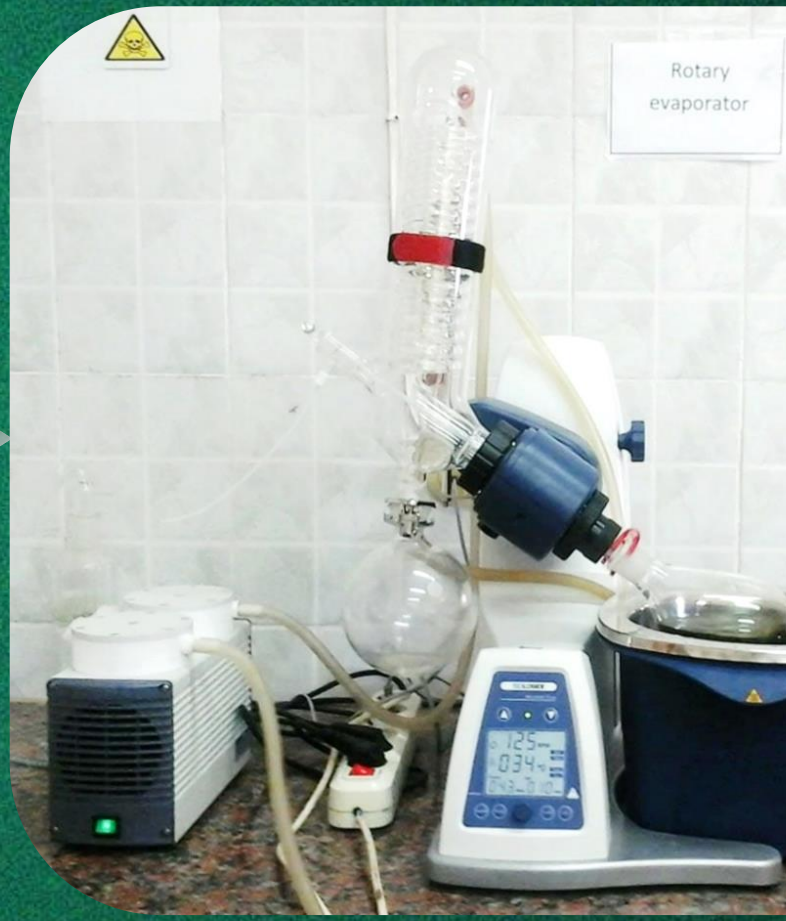
Grinding of the dried leaves

Methods

Method 03

Preparation of Four Extracts from Dried Leaves and Cones of Studied Plants

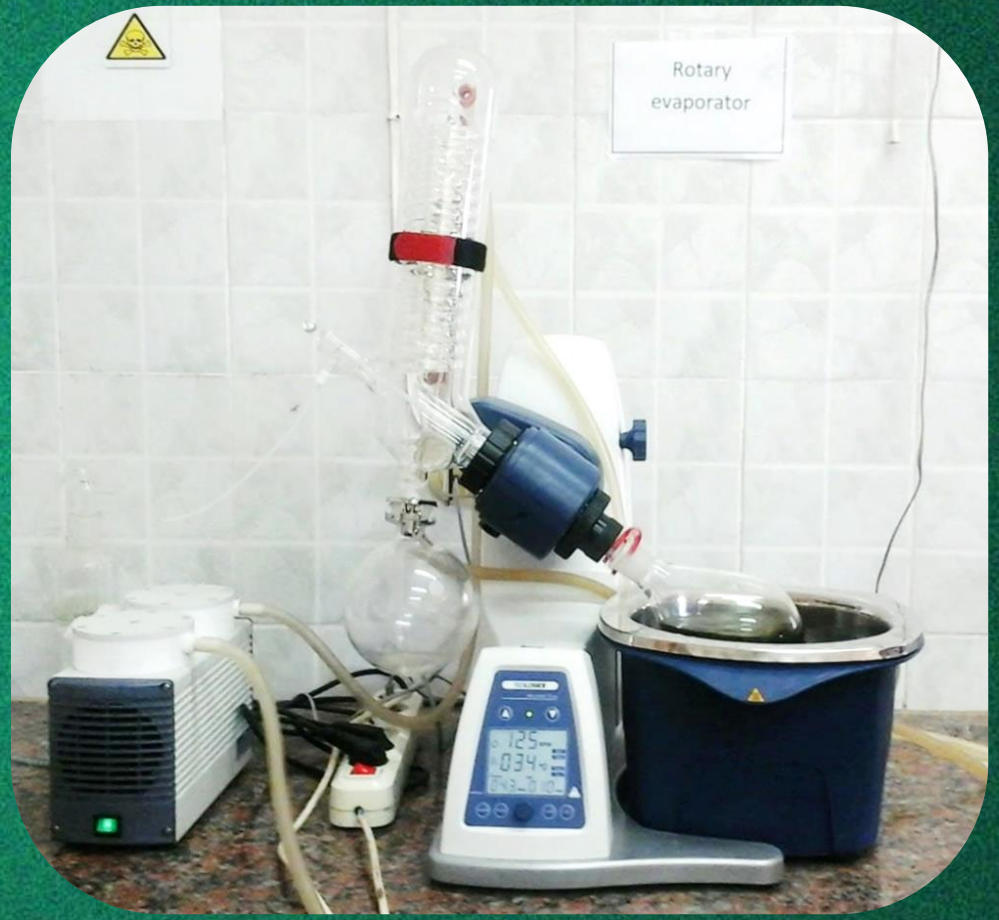




Methods

Method 03

Preparation of Four Extracts from Dried Leaves and Cones of Studied Plants

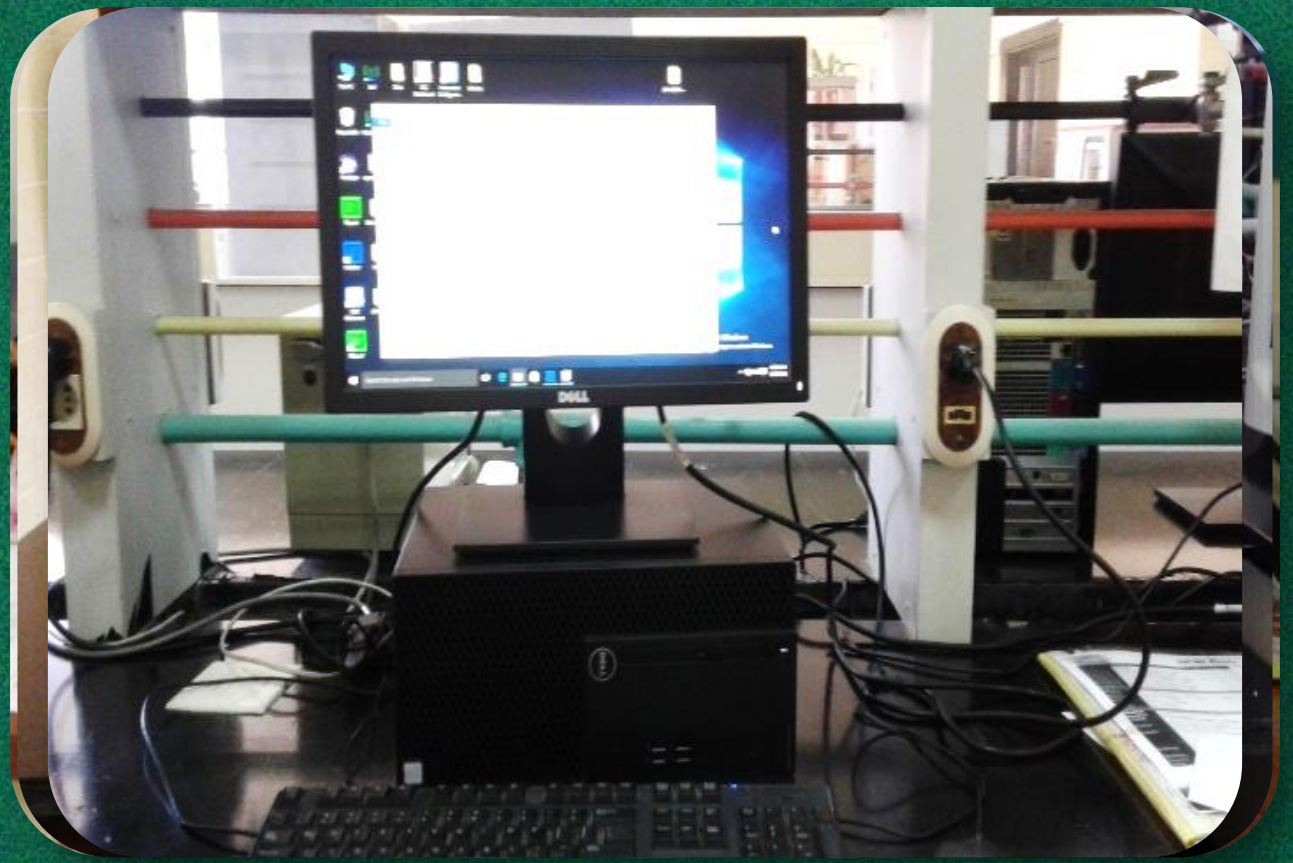


Methods

Method 03

Preparation of Four Extracts from Dried Leaves and Cones of Studied Plants

Characterization



Methods

Method
04

Maintaining the Mosquito by Rearing the Culture of *Culex Pipiens*

Methods

Method 04

Maintaining the Mosquito by Rearing the Culture of *Culex Pipiens*

Methods

Method
05

Dose Response Bioassay Separately of the all Preparations, Extracts and Chemical Insecticide

Methods

Method 05

Dose Response Bioassay Separately of the all Preparations, and Extract

50 ml Dechlorinated Water
+ 10 Larvae

Each control and Treatment were Replicated Three Times

1 | 0, 20, 30, 40, 50 and 60 ppm PDG

2 | 0, 25, 50, 75, 100 and 150 ppm EO

3 | 0, 120, 140, 160, 180 & 200 ppm E

Methods

Method
05

Dose Response Bioassay Separately of the all Preparations, and Extract

Methods

Method
06

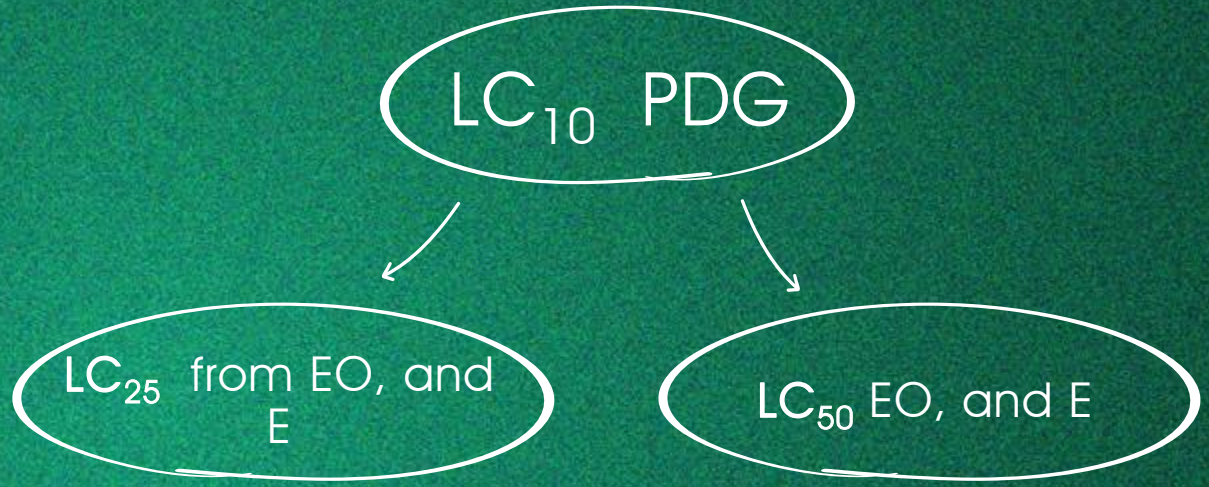
Investigation for the synergistic effect of prodigiosin with essential oil, and extract individually as mosquito larvicidal potential

Methods

Method 06

Investigation for the synergistic effect of prodigiosin with essential oil, and extract individually as mosquito larvicidal potential

50 ml Dechlorinated Water
+ 10 Larvae



The mortality of the larvae was recorded after an hour, 3, 6,12, 24, 48, 72 and 96 hours

Methods

Method 07

Investigating the Mode of Action of PDG, Essential Oil and Extract for Mosquito Larvicidal Potentially

Preparation of ‘Whole Body Homogenates’

Methods

Method 07

Investigating the Mode of Action of PDG, Essential Oil and Extracts for Mosquito Larvicidal Potentially **Preparation of 'Whole Body Homogenates'**

01 **Untreated Larvae** (20-30 larvae) with:

02 **Treated Larvae** (20-30 larvae) with:

- **Prodigiosin at LC_{10}** concentration
- **Essential oil, and extracts treated larvae** at the LC_{25} concentration
- **Essentia oil, and extract treated larvae** at the LC_{50} concentration
- **PDG Treated** at LC_{10} in combination with EO as well as in combination with extract treated at LC_{25} and LC_{50}

After 24 hour

Were Washed with Distilled Water to Remove the Adhering Water

Methods

Method 07

Investigating the Mode of Action of PDG, Essential Oil and Extract for Mosquito Larvicidal Potentially

Preparation of 'Whole Body Homogenates'

01 **Treated Larvae** (20-30 larvae) with:

02 **The larvae then pooled and homogenized in** Eppendorf tubes **(held in ice)** using a Teflon hand homogenizer in **1 ml of 0.9% w/v saline** for eventual estimation of total protein, acetylcholine esterase.

03 Homogenates were centrifuged **at 4,000 rpm for 15 min at 4 °C** in a cooling centrifuge

04 **The clear supernatants** were kept **at -80 °C until** use for biochemical analysis

Methods

Method 07

Investigating the Mode of Action of PDG, Essential Oil and Extract for Mosquito Larvicidal Potentially

Preparation of 'Whole Body Homogenates'

Eppendorf Cooling Centrifuge

Methods

Method 07

Investigating the Mode of Action of PDG, Essential Oil and Extract for Mosquito Larvicidal Potentially

Preparation of 'Whole Body Homogenates'

Anticholinesterase Activity

Total Protein Activity

Methods

Method 07

Investigating the Mode of Action of PDG, Essential Oil and Extract for Mosquito Larvicidal Potentially

pH Determination by Bromothymol Blue Dye

pH determination
in the 3rd Stage Larval Midgut

Methods

Method
08

Probit Analysis for Calculating the Lethal Concentration of PDG, Essential Oils and Dry Extract

Methods

Method 08

Probit Analysis for Calculating the Lethal Concentration of PDG, Spinosad Essential Oils and Dry Extracts

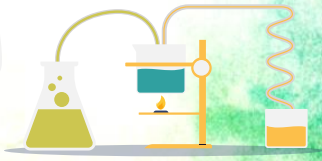
Mortality Rate was Calculated using Abbott's Formula

The dose–response data was Analyzed by Probit Regression

The Lethal Concentrations in ppm **(LC_{10}, LC_{25} and LC_{50}),** and the **95% confidence** intervals [upper confidence limit (UCL) and lower confidence limit (LCL) were Calculated

Results

CHAPTER FOUR



1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

Results

1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

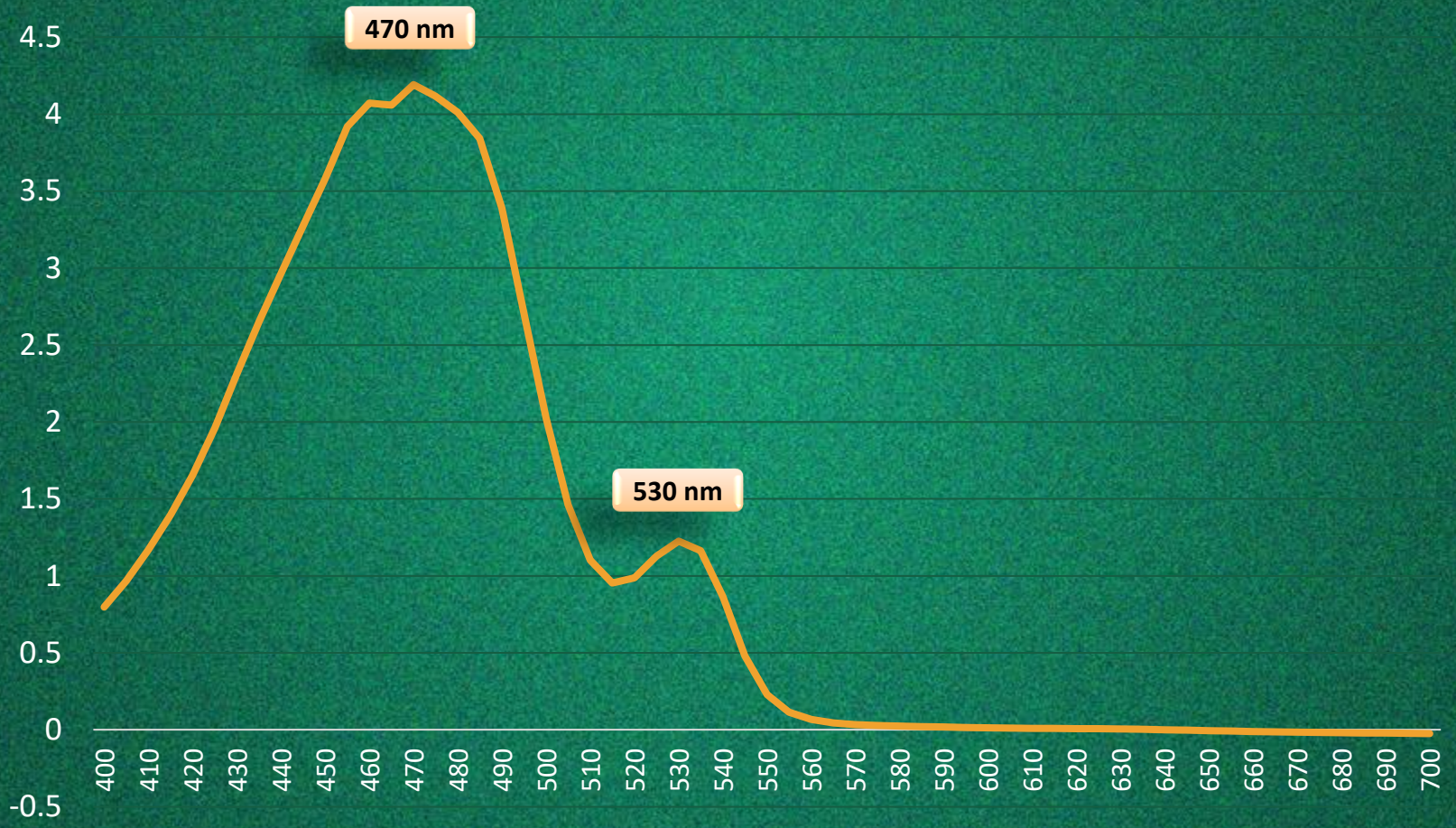
Shows the UV-visible spectrophotometry in the range 400-700 nm for the crude PDG

A) PDG-batch Scale

1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

Shows the UV-visible spectrophotometry in the range 400-700 nm for the crude PDG

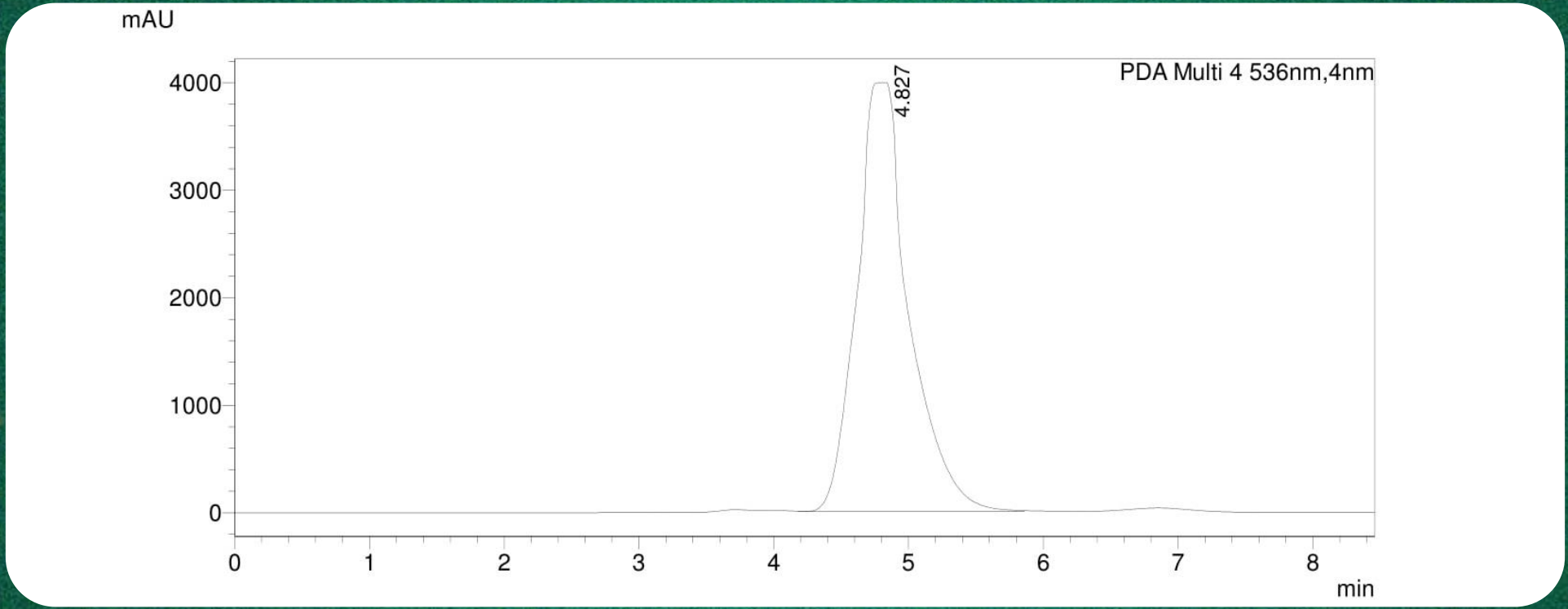


B) PDG-Fermenter (Bioreactor)

1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

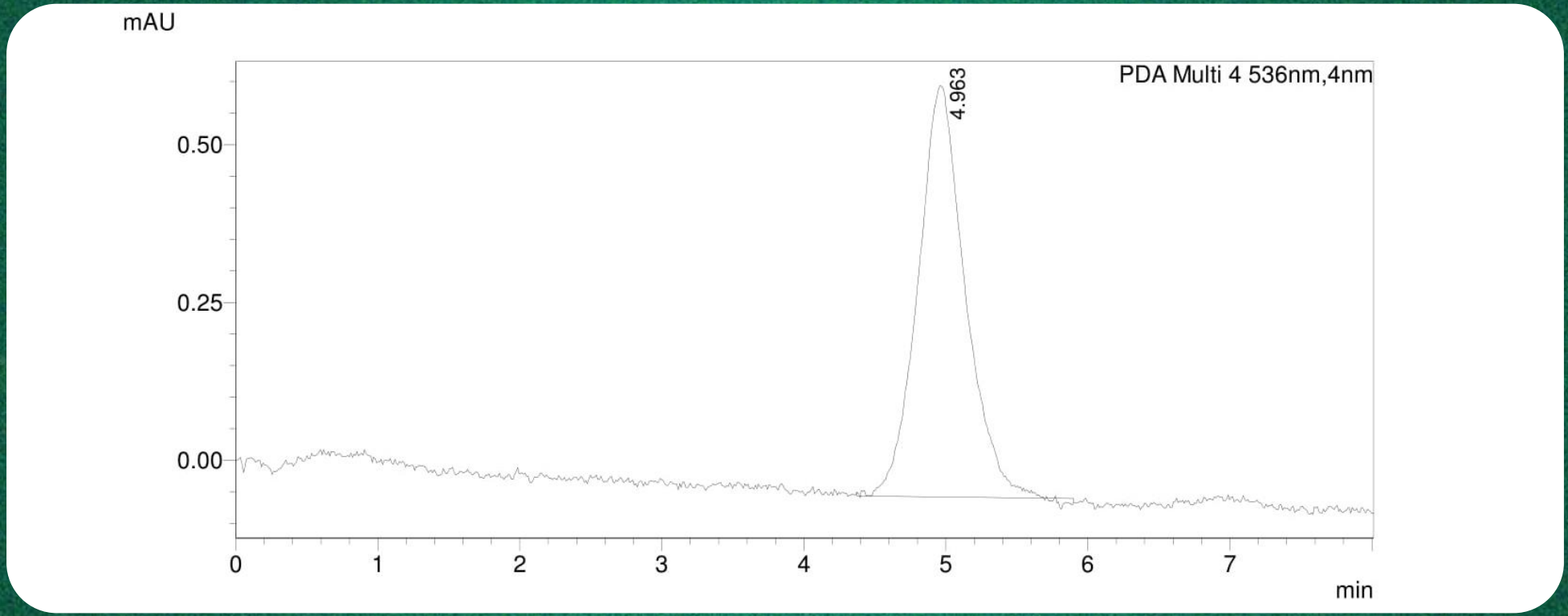
HPLC for the prepared and the standard PDG



A) Purified Red Pigment

1 Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

HPLC for the prepared and the standard PDG

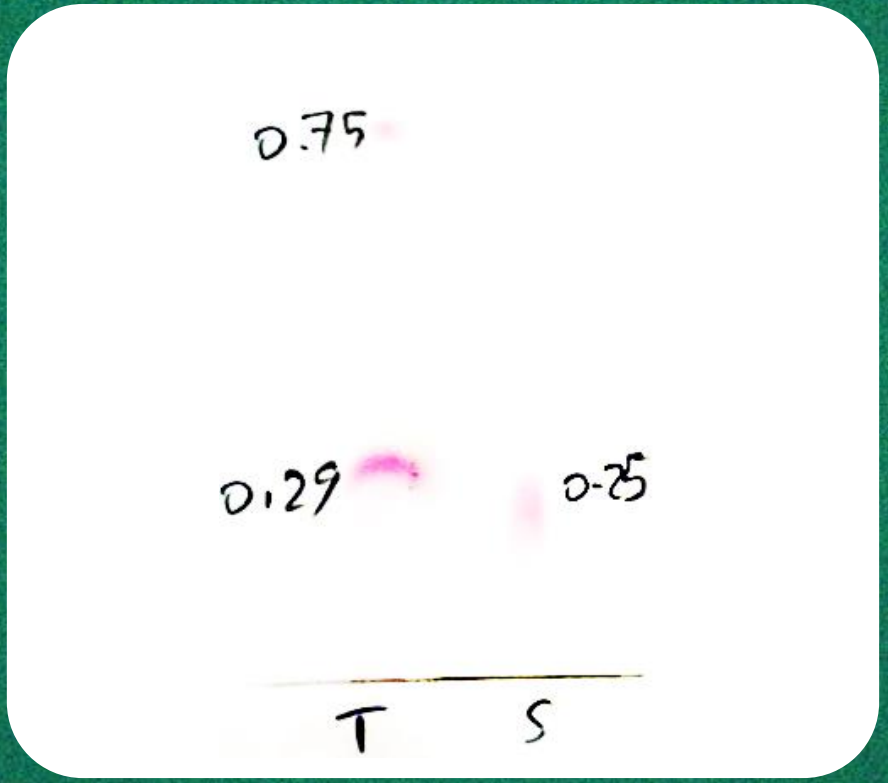


B) Standard PDG

1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

Trials of TLC application to determine the best mobile phase

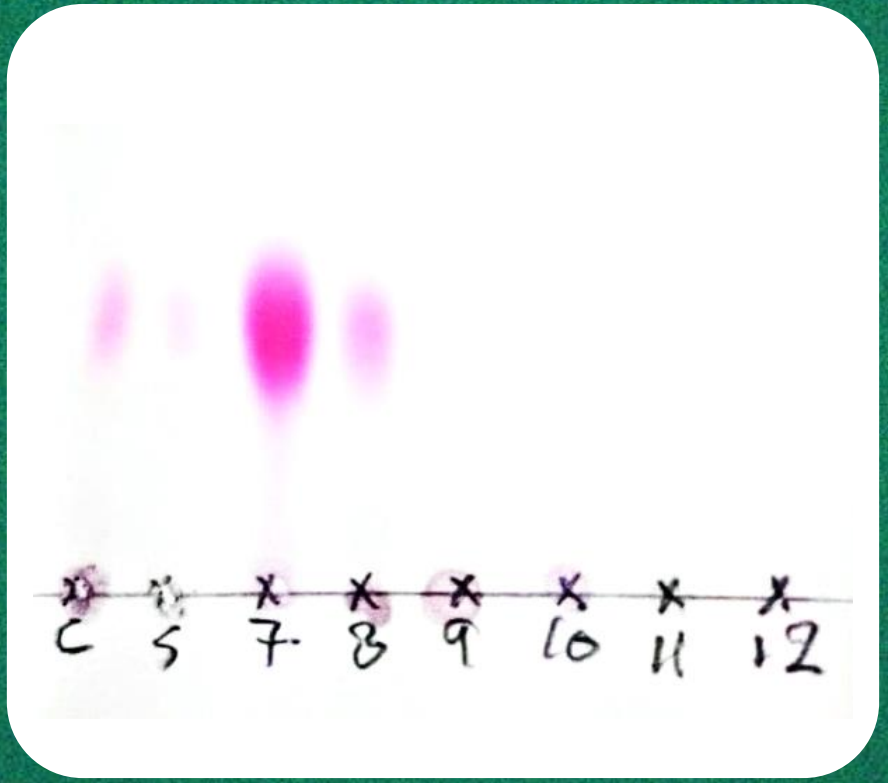


n-hexane: Ethyl acetate 2: 1

1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

Application of the purified PDG fractions and the standard on TLC

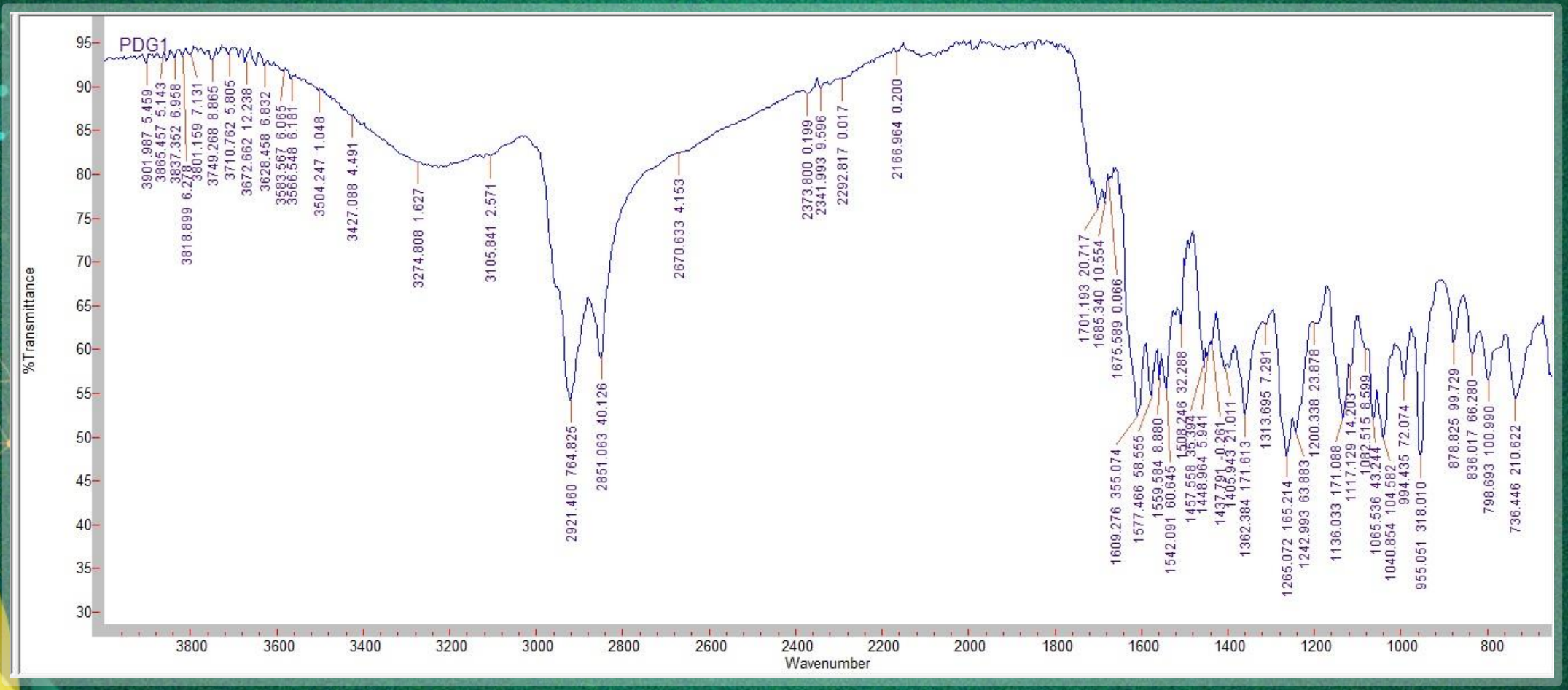


n-hexane: Ethyl acetate 2: 1

Pigment

1 Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

FT-IR analysis of the purified Red Pigment

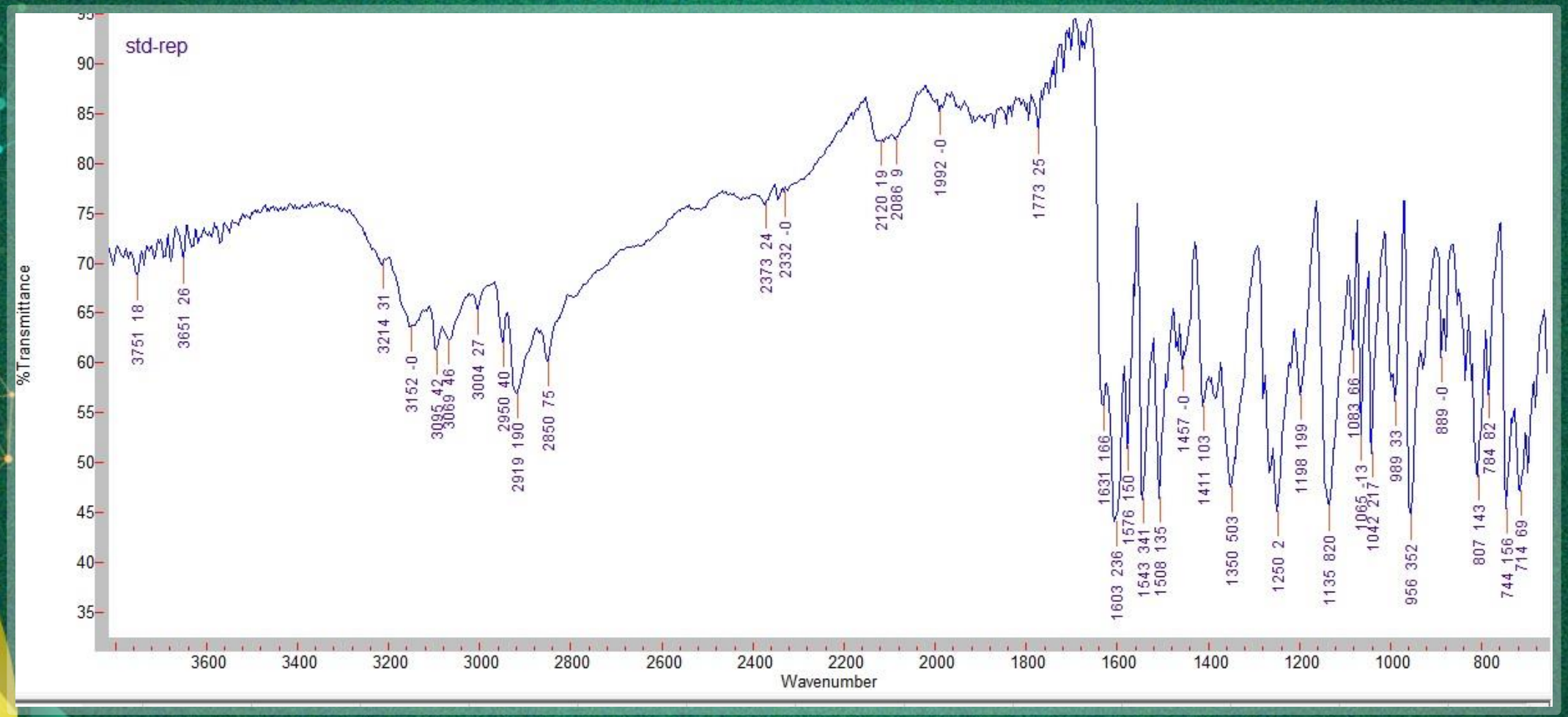


1

Isolation, Purification and Characterization of Prodigiosin from *Serratia marcescens*

Standard

FT-IR analysis of the PDG Standard



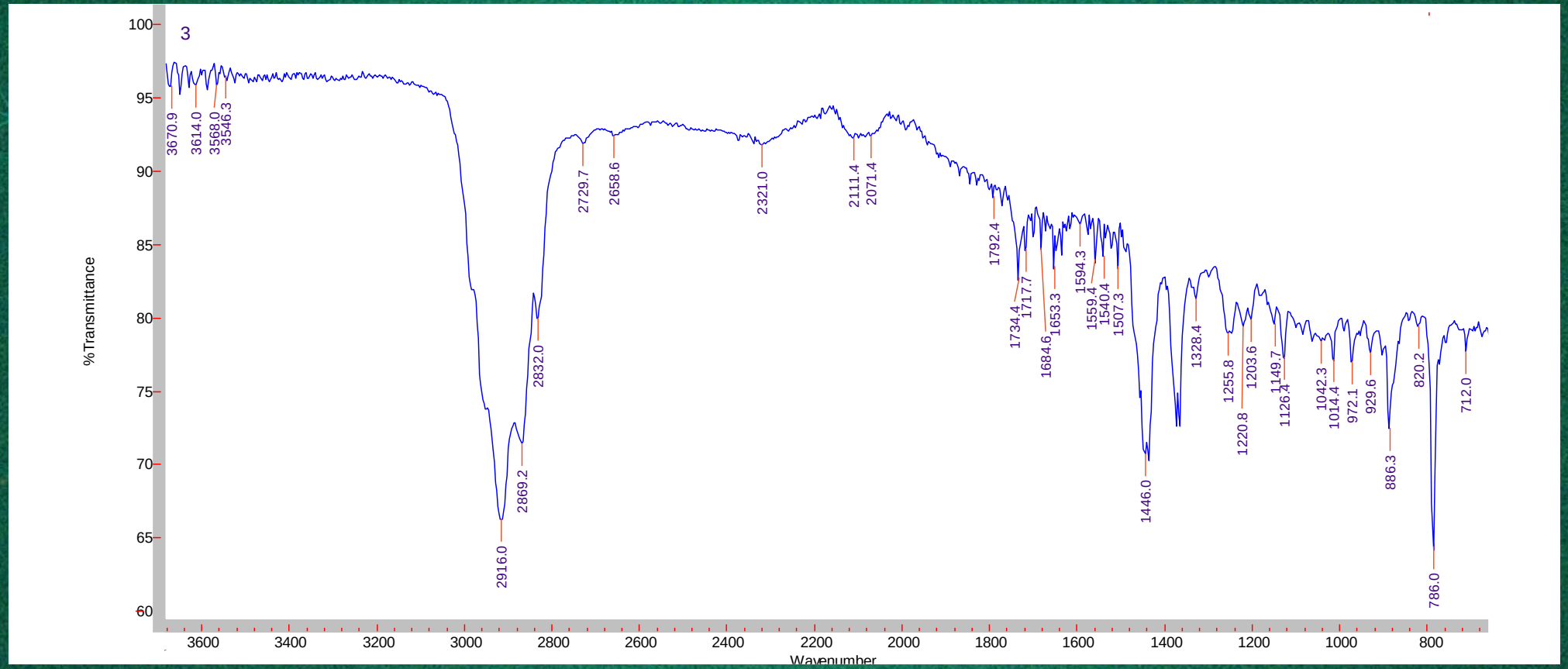
2

Preparation and Characterization of Four Essential Oils from Fresh Leaves and Cones of Studied Plants

Results

2 Preparation and Characterization of Four Essential Oils from Fresh Leaves of *Thuja orientalis*

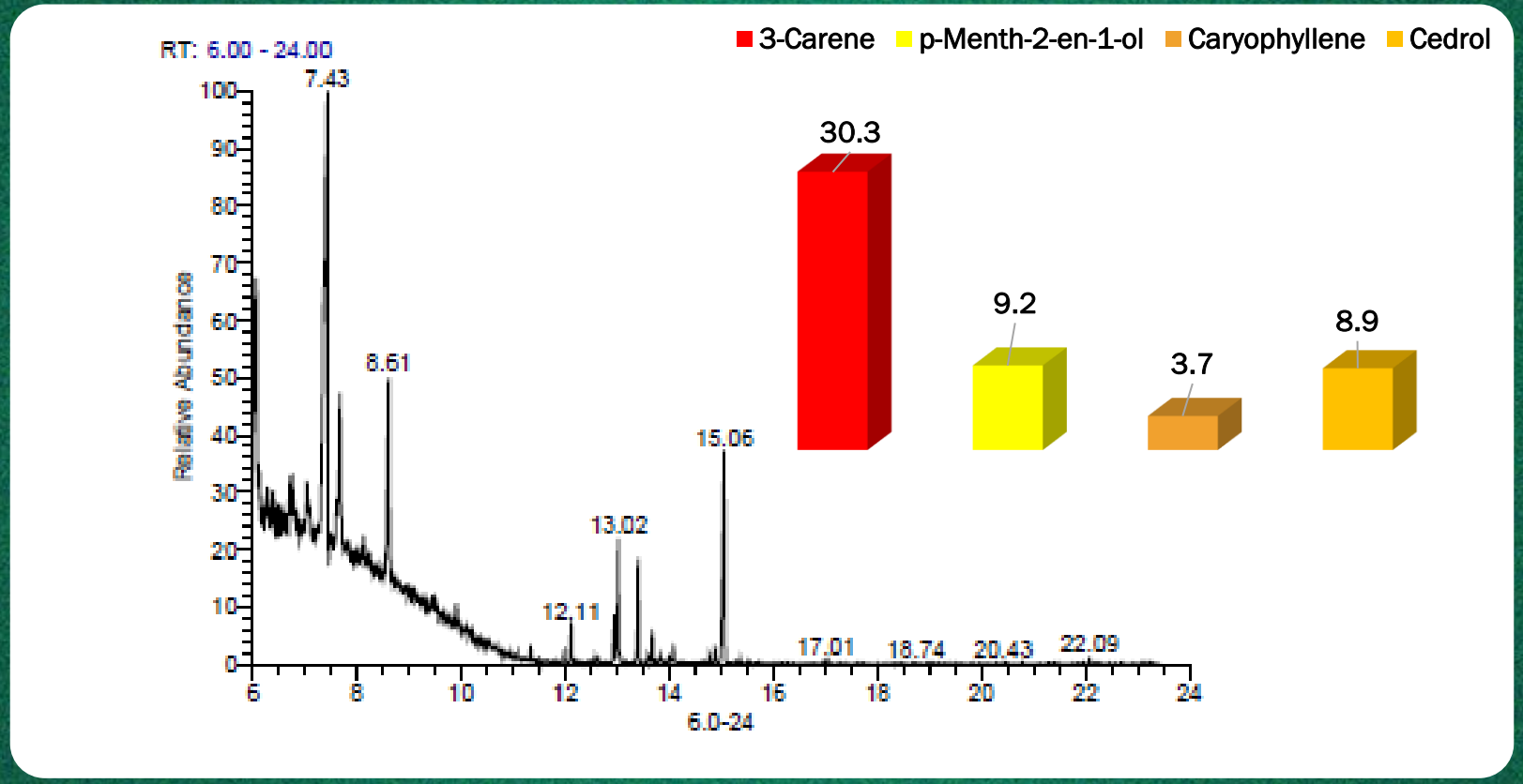
FT-IR Spectra



FT-IR analysis for the Essential Oil Prepared from **Fresh Leaves**

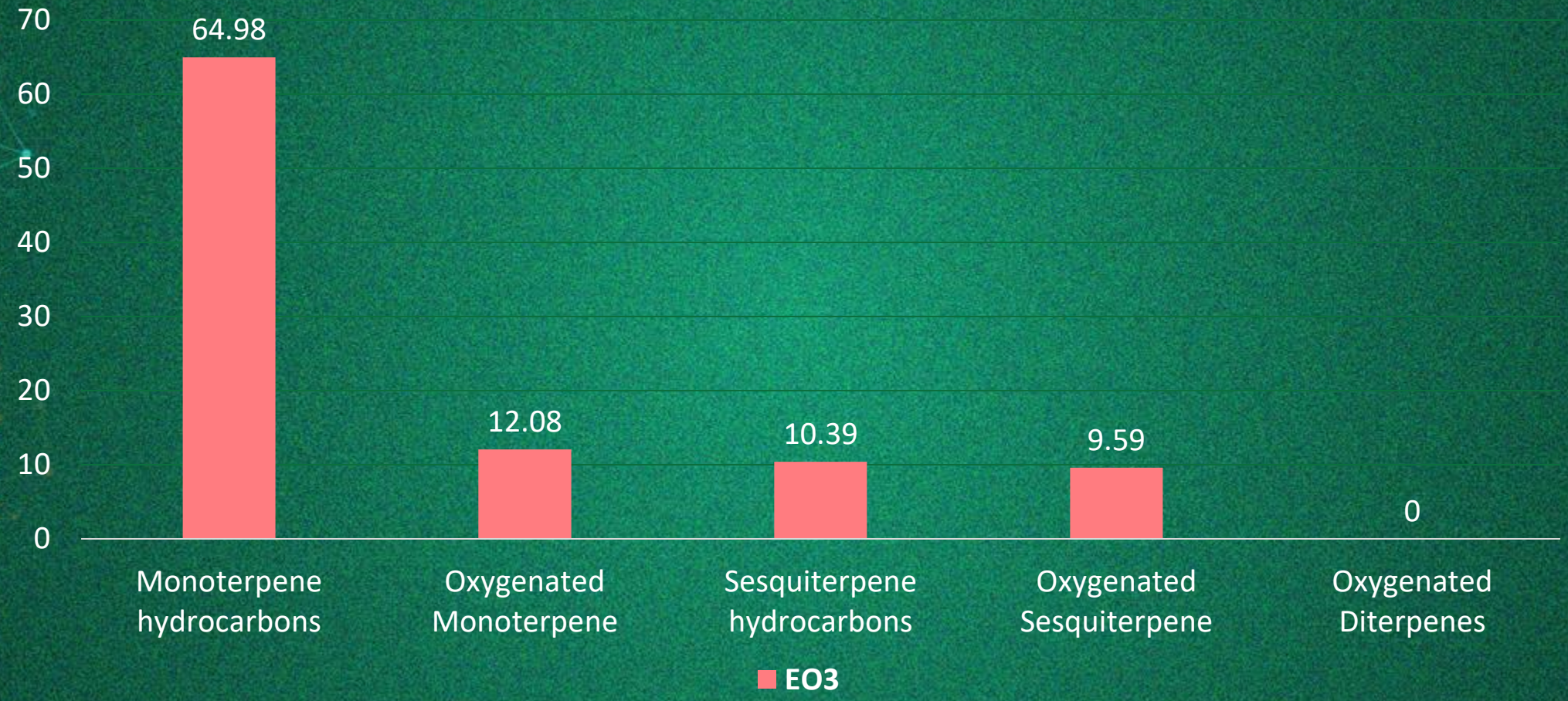
2 Preparation and Characterization of Four Essential Oils from Fresh Leaves of *Thuja orientalis*

GC-MS



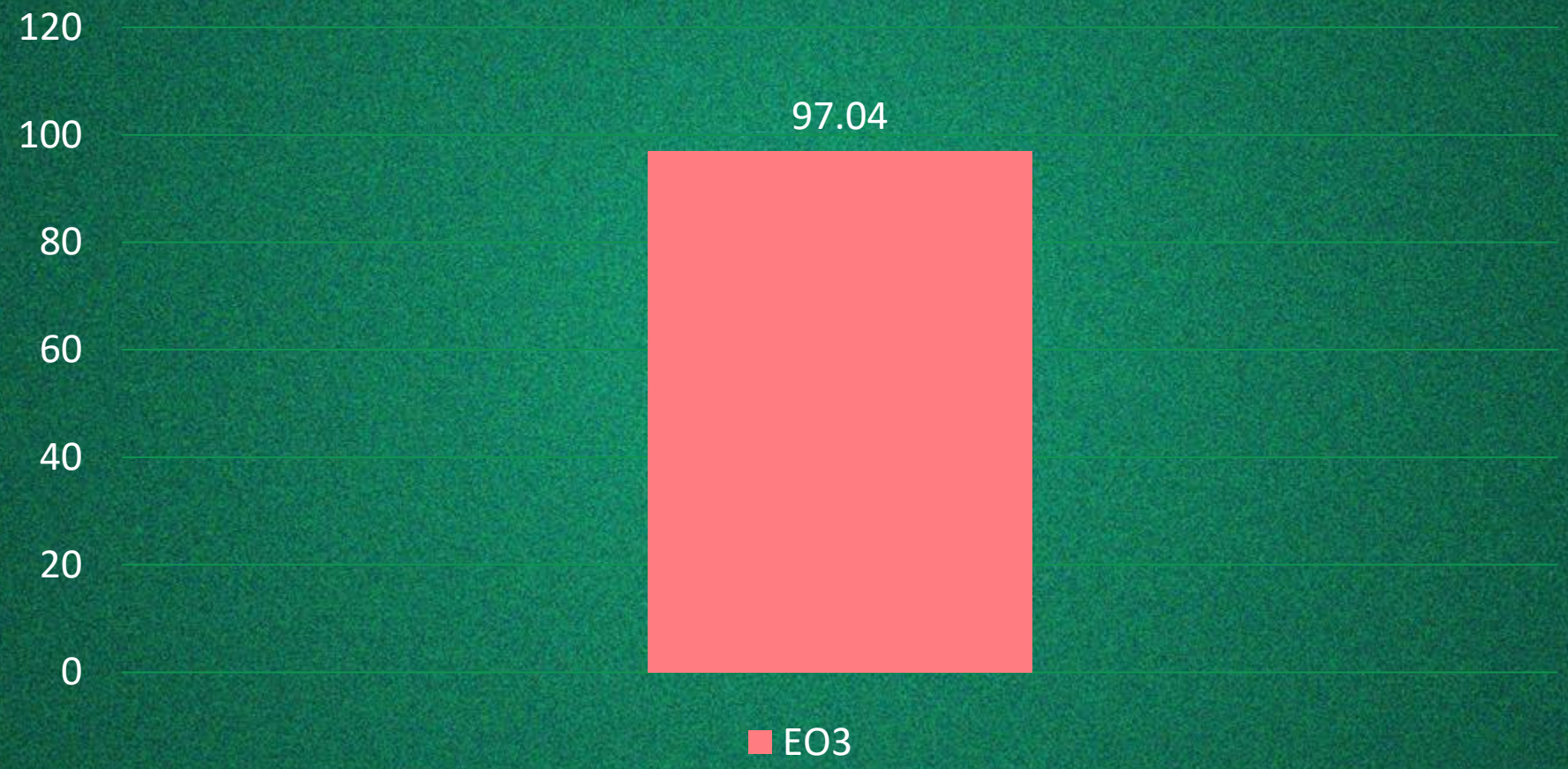
GC-MS analysis for the essential oil prepared from **fresh leaves of *Thuja orientalis***

2 Preparation and Characterization of Four Essential Oils from Fresh Leaves of *Thuja orientalis*



GC-MS analysis for the essential oil prepared from **fresh leaves of *Thuja orientalis***

2 Preparation and Characterization of Four Essential Oils from Fresh Leaves of *Thuja orientalis*



Total percentage of Constituents revealed by GC-MS in *Thuja orientalis*

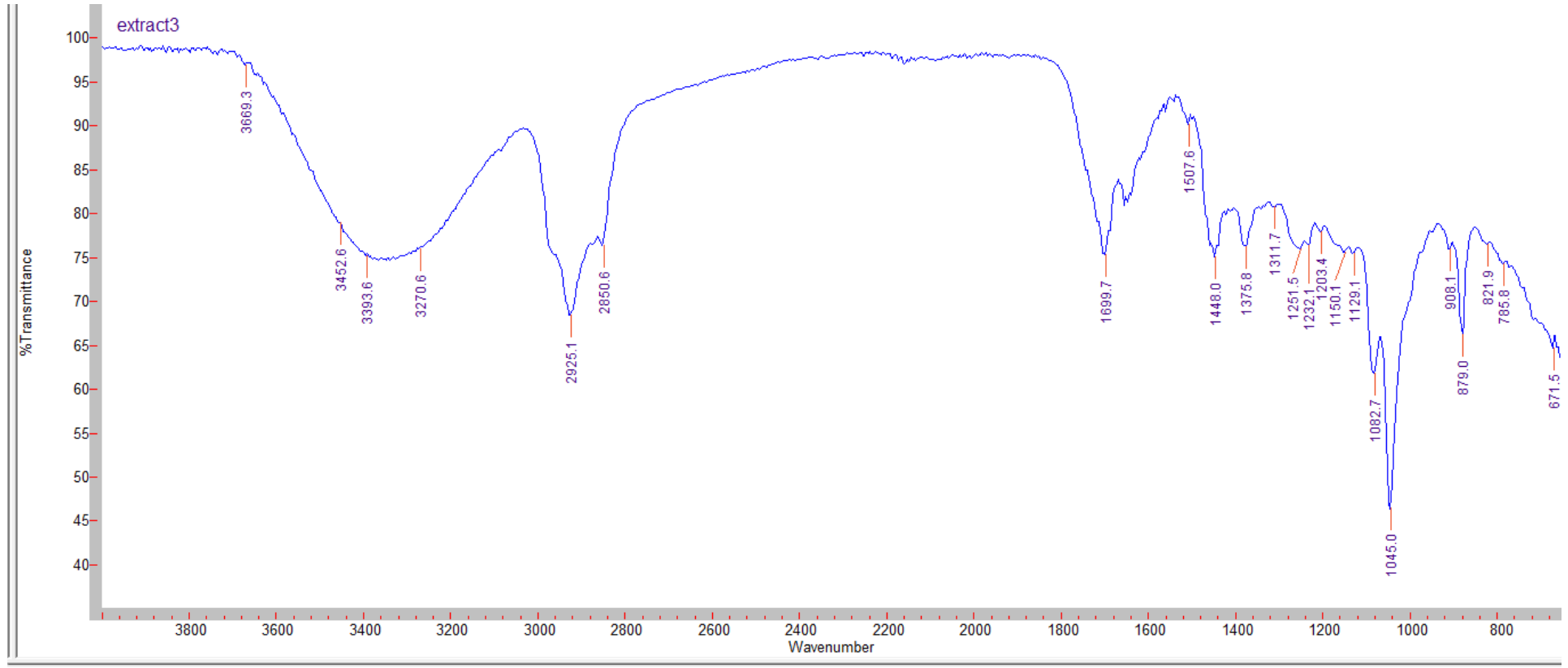
3

Preparation and Characterization of Four Crude Extracts from Dried Leaves of Studied Plants

Results

3 Preparation and Characterization of Four Crude Extracts from Dried Leaves of Studied Plants

FT-IR analysis for the extract of dry leaves of *Thuja orientalis* (E3)

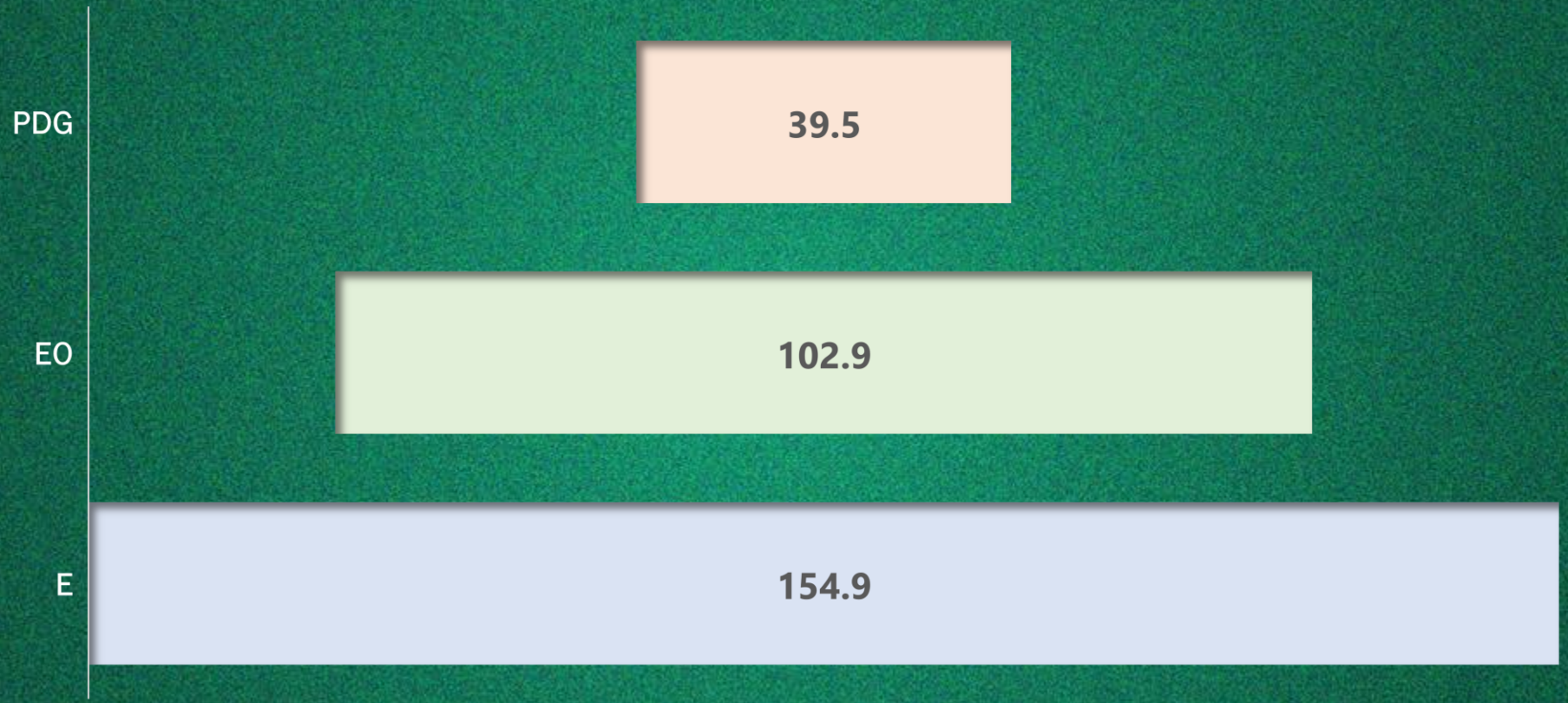


4

Dose Response Bioassay Separately of all Preparations, and Extract

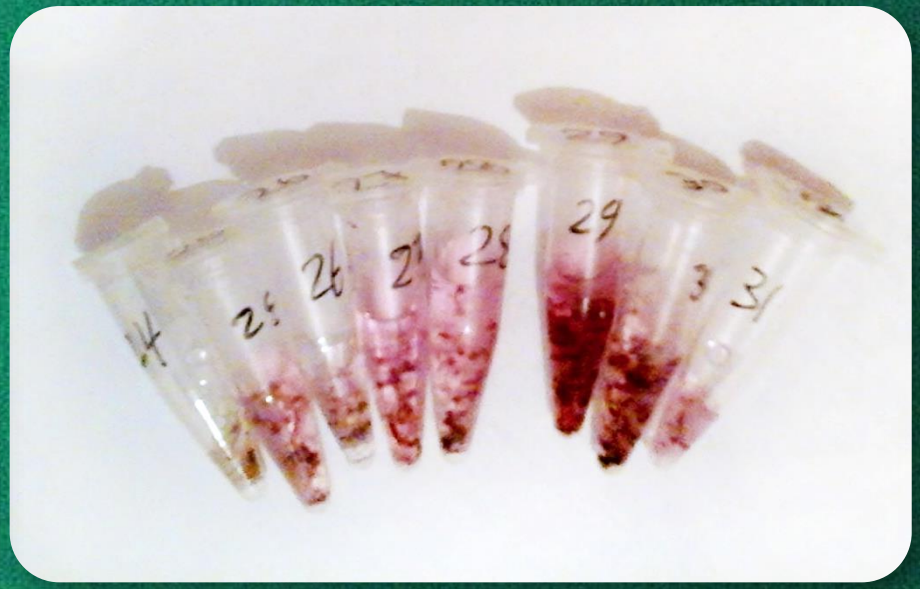
Results

4 Dose Response Bioassay Separately of all Preparations, and Extract



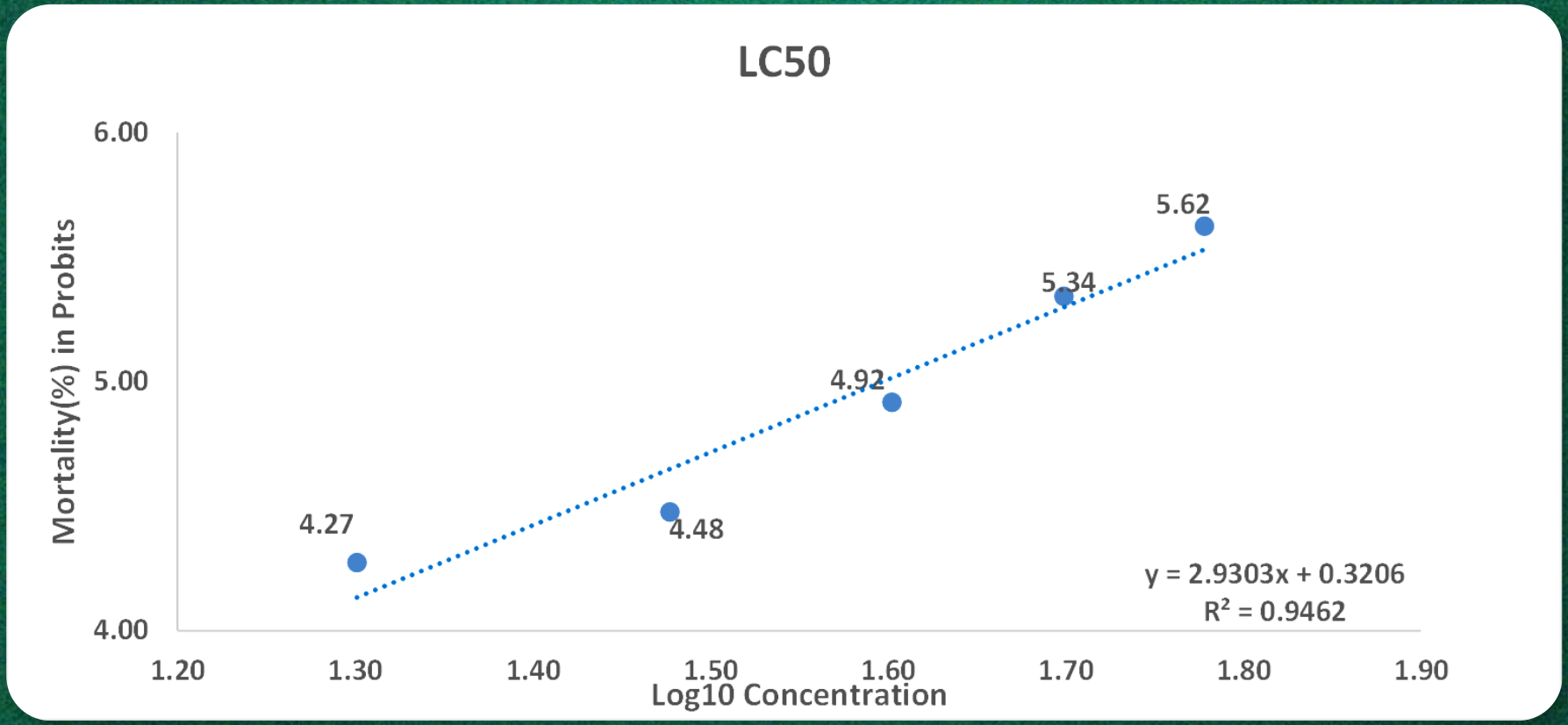
Larvicidal activity of the studied preparations after 24 hours against the **3rd larval stage of *Cx. pipiens***

4 Dose Response Bioassay Separately of all Preparations, and Extract



Dead Larvae after Treatment with PDG & E.O

4 Dose Response Bioassay Separately of all Preparations, and Extracts



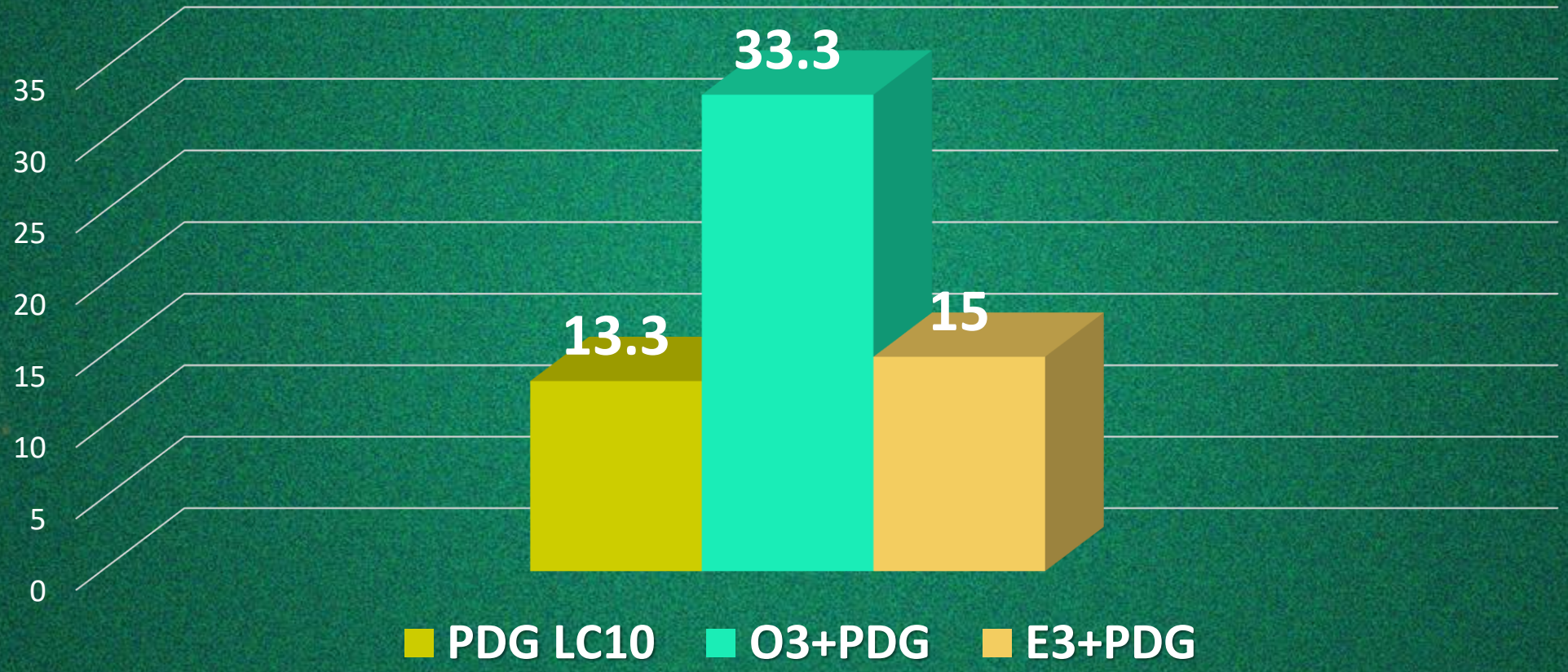
The concentration of PDG at LC50 after 24 Hours

5

Synergistic Effect of PDG with the natural Pds Individually as a Mosquito Larvicidal Potential after 24 hrs.

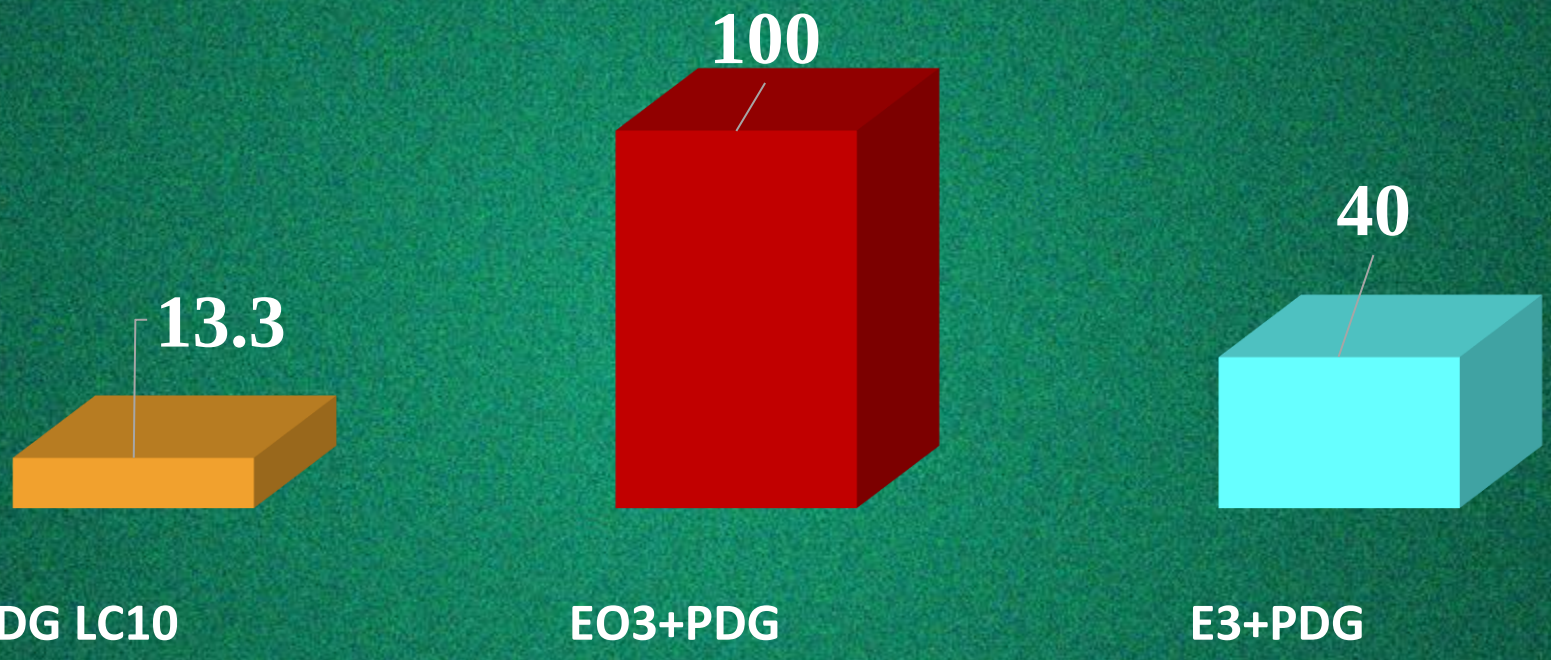
Results

5 Synergistic Effect of PDG with the natural Pds Individually as a Mosquito Larvicidal Potential after 24 hrs.



Synergistic Larvicidal activity of the LC_{10} of PDG with LC_{25} of Oil, and the extract Larvicide ***(Spinosad)*** after 24 hrs.

5 Synergistic Effect of PDG with the natural Pds Individually as a Mosquito Larvicidal Potential after 24 hrs.



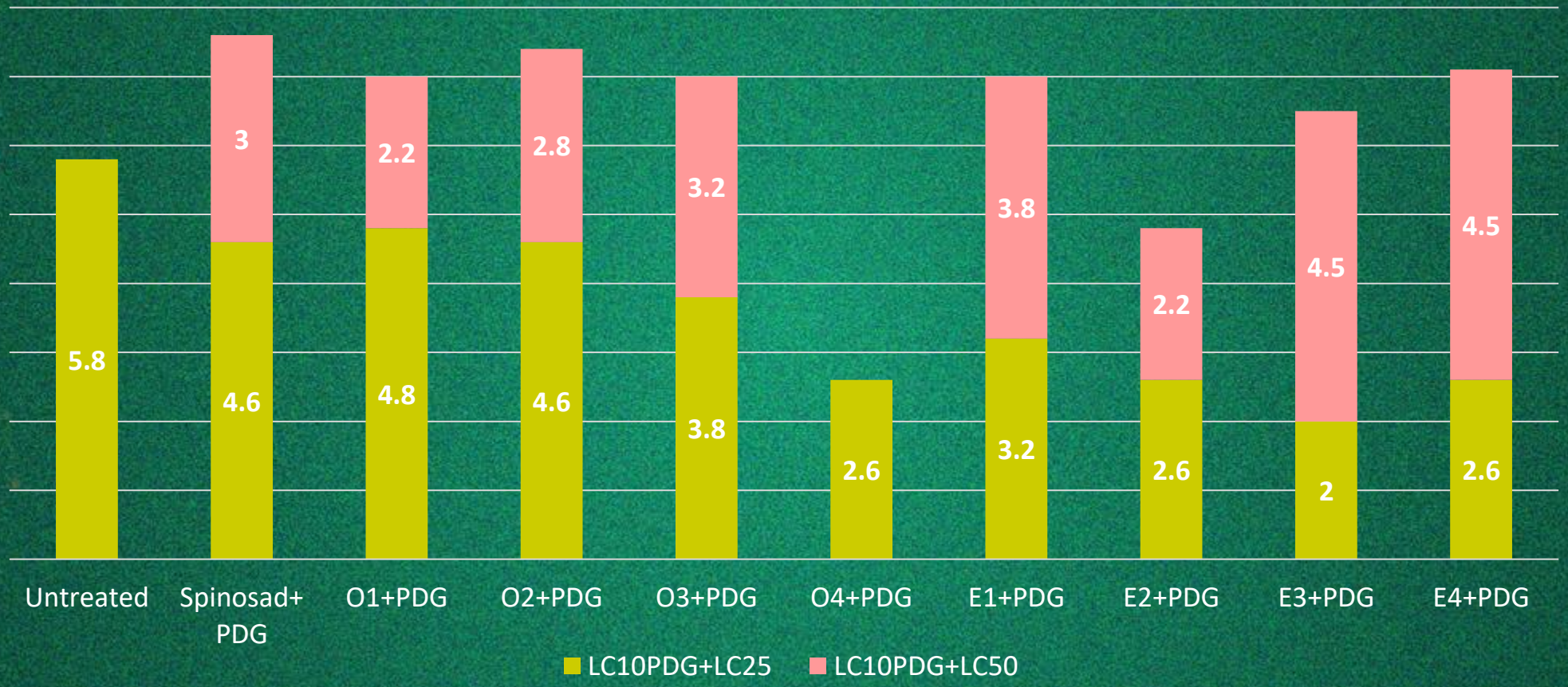
Synergistic Larvicidal activity of the LC_{10} of PDG with LC_{50} of Oils, extracts and the Chemical Larvicide ***(Spinosad)*** after 24 hrs.

6

Investigating the Mode of Action of PDG, Essential Oil, and the extract for Mosquito Larvicidal Potentially

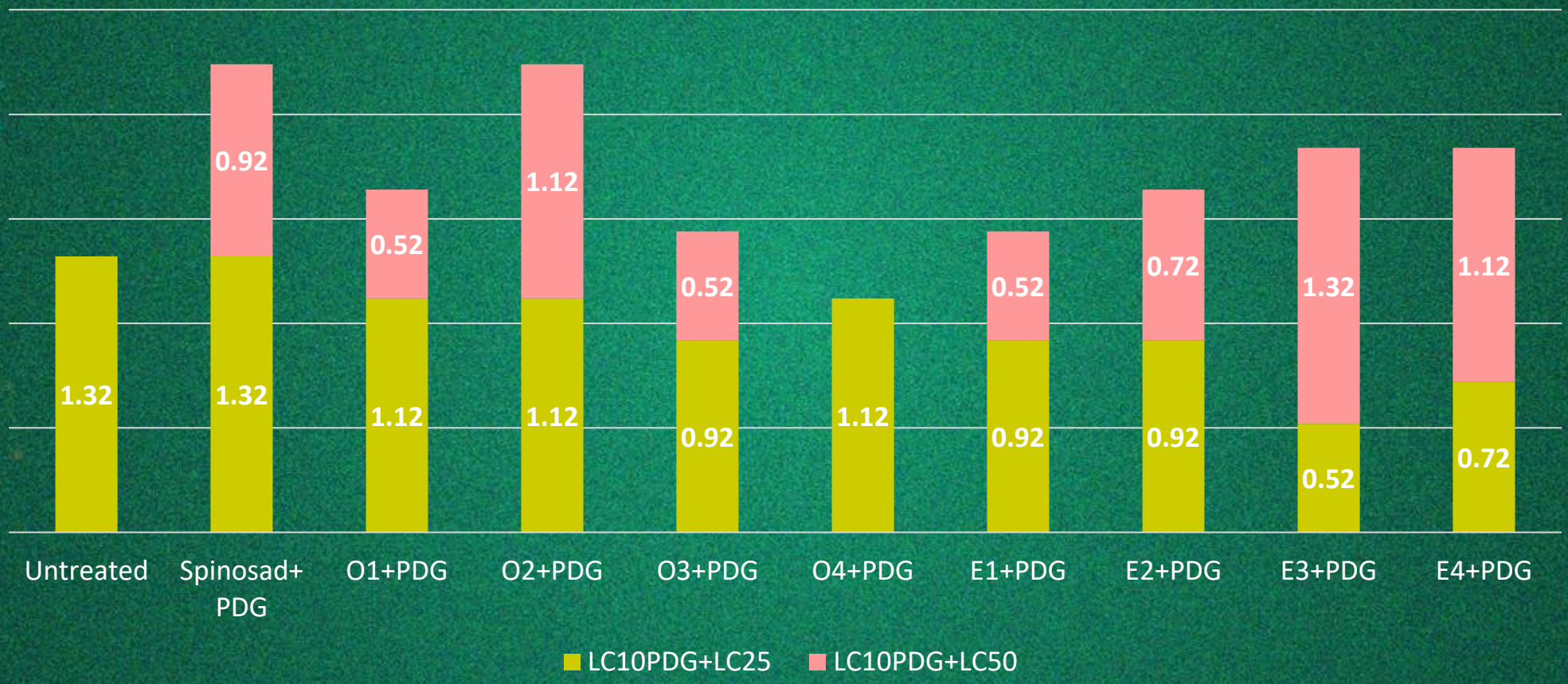
Results

6 Investigating the Mode of Action of PDG, Essential Oil, and extracts for Mosquito Larvicidal Potentially



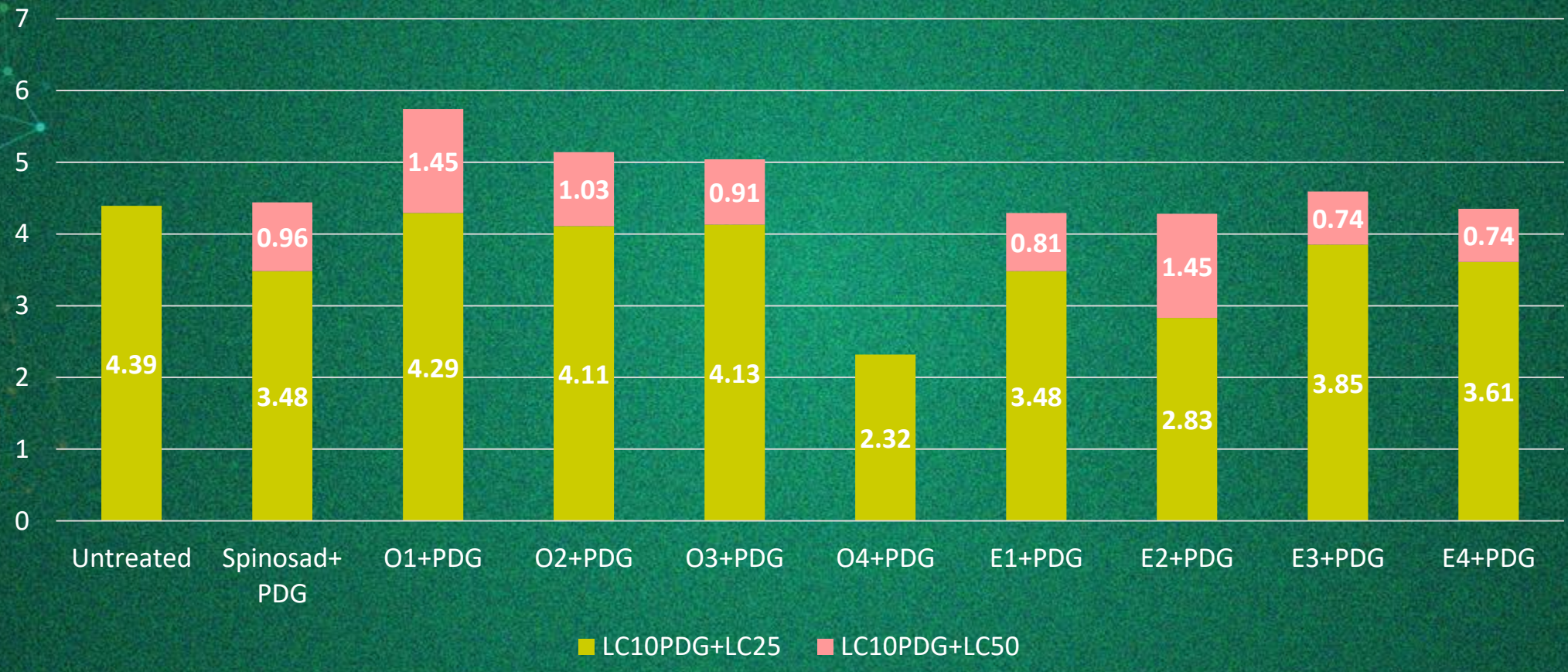
AChE Arbitrary Activity unit/gm Tissue (%)

6 Investigating the Mode of Action of PDG, Volatile Oils, extracts and Spinosad for Mosquito Larvicidal Potentially



Total Protein in mg /gm tissue (%)[3]

6 Investigating the Mode of Action of PDG, Volatile Oils, extracts and Spinosad for Mosquito Larvicidal Potentially



AChE Arbitrary Specific Activity² (%)³

6 Investigating the Mode of Action of PDG, Essential Oil, and extract for Mosquito Larvicidal Potentially

pH Indicator

Midgut of Untreated 3rd Larval Stage showed alkaline media by using Bromothymol blue dye that act as pH indicator **(1.6 X)**

6 Investigating the Mode of Action of PDG, Essential Oil, and extract for Mosquito Larvicidal Potentially

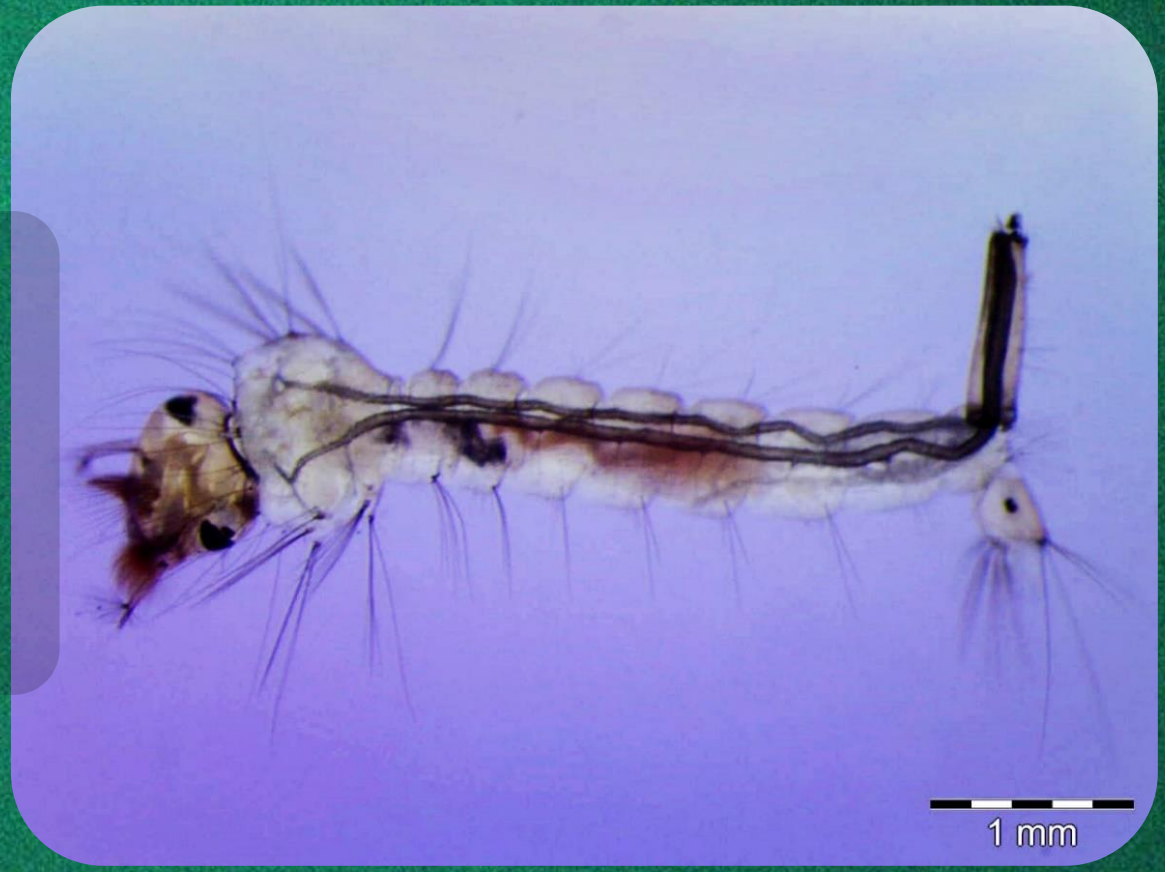
pH Indicator

Midgut of PDG Treated 3rd Larval Stage showed acidic media by using Bromothymol blue dye that act as pH indicator **(1.6 X)**

6 Investigating the Mode of Action of PDG, Essential Oil, and extract for Mosquito Larvicidal Potentially

pH Indicator

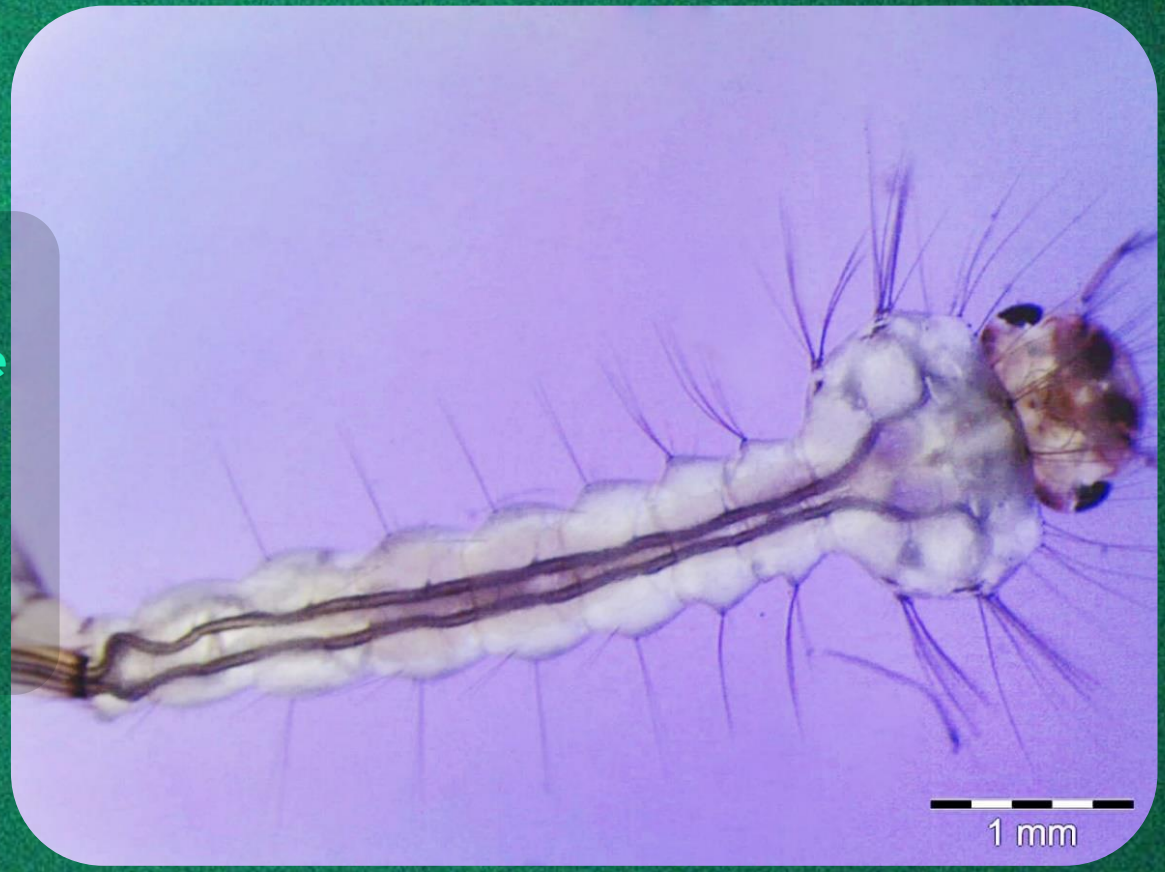
Midgut of E.O Treated 3rd Larval Stage showed acidic media by using Bromothymol blue dye that act as pH indicator **(1.6 X)**



6 Investigating the Mode of Action of PDG, Essential Oils and extract for Mosquito Larvicidal Potentially

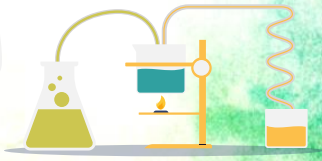
pH Indicator

Midgut of PDG+E.O Treated 3rd Larval Stage showed acidic media by using Bromothymol blue dye that act as pH indicator **(1.6 X)**



Conclusion and Recommendation

CHAPTER FIVE



01

Purified rodigiosin showed the lowest LC_{50} followed by crude essential oils of ***Thuja orientalis*** leaves and its **Crude extract**

02

The combination between LC_{10} of prodigiosin and **LC_{50}** of ***T. orientalis*** **E.O**, showed the highest **synergistic effect (100%)**

03

The treated 3rd larval *Cx. pipiens* showed reduction in the acetylcholine esterase and total protein content as compared to the **untreated ones**

04

The midgut of the treated 3rd larval *Cx. pipiens* showed **acidic medium** in contrast the untreated ones showed **alkaline medium** by stereomicroscope after using bromothymol blue dye

05

The present study recommended that the combination of prodigiosin and E.O. from ***T. orientalis*** leaves is a promising potent larvicid against the 3rd larval ***Cx. pipiens.***

Thank you