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Anti-microbial Activity of 3 Different Species of Tulsi Found in Chhattisgarh against Alcaligenes Faecalis & Salmonella Enterica

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Abstract

Tulsi (*Ocimum* spp.), an aromatic plant belongs to the family, Lamiaceae. Tulsi can be grouped to two broad categories, namely holy basil (*Ocimum sanctum*) and mediterranean basil (*Ocimum basilicum*). The present study was carried out to compare phytochemical and antimicrobial activity of three Tulsi species, namely Rama Tulsi (*Ocimum sanctum*), Krishna Tulsi (*Ocimum tenuiflorum*), and mediterranean basil (*Ocimum basilicum*). These tulsi species can be found in different agro-climatic zones of Chhattisgarh. For anti-microbial activity against bacteria different extractions were made out of all three species (Petroleum ether extract, Ester extract, methanol extract, chloroform extract and formaldehyde extract). Two methods for anti-microbial activity testing were done i.e. well diffusion and disc diffusion method. Results showed that among all species of tulsi and two bacterial strains, *Ocimum tenuiflorum* showed maximum zone of inhibition with petroleum ether, ester and methanol extracts.

Keywords: Antimicrobial activity, Solvent extraction, Salmonella enterica, Alcalagens faecalis.

INTRODUCTION

Holy Basil also known as Tulsi (*Ocimum sanctum*), an aromatic herb has healing and curative properties. It is a sacred herb in India and is grown in all houses, temples, gardens, etc. It

grows to a height of 1-1.5 meters and has quadrangular branches. Leaves grow opposite to each other with a length 2-4 cm, leaf margins are either entire or toothed, and possesses hair on both the surfaces. Tiny, purple flowers grow on Tulsi and inflorescence is 12-14 cm in length. The fruits which grow on Tulsi are small and smooth nuts. Tulsi is used in treatment of a number of diseases like mental illness, cough and fever, gut diseases, bone and joint problems, eye diseases and other optic problems, ringworm, insect bite, snake bite and scorpion bite and malaria.

Tulsi has antimicrobial activities against many pathogens and can be used as mouth wash agent, for wound healing, and preservation of food stuff. Tulsi is antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and can be used also for killing mosquitoes. It has anti-oxidants and can be used as anti-cataract agent, anti-inflammatory agent, as well as protects from chemicals and radiations, good for the liver and nerves and heart, anticancerous agent, protects the immune system, central nervous system and memory its anti-asthma and thyroid, and solves fertility issues.

Tulsi is known to help protect vital human organs and cells against various types of chemical strains from the prevalent industrial pollution and fossil fuels emissions and physical strains from extended physical exhaustion and restraint due to various physical problems and noise exposure to excessive and loud ones. The present study involved comparative analysis of antimicrobial activity of three Tulsi species, namely Rama Tulsi (*Ocimum sanctum*), Krishna Tulsi (*Ocimum tenuiflorum*), and mediterranean basil (*Ocimum basilicum*).

MATERIALS AND METHODS

The different varieties of Tulsi were procured from different agro-climatic zones of Chhattisgarh. The spp. were subjected to different extraction methods viz. petroleum ether, ester, methanol, chloroform and formaldehyde. These extracts were used for anti-microbial activity by using two methods like well diffusion and disc diffusion method. Extracts were tested against two bacterial strains *Salmonella enterica* and *Alcaligens faecalis*. All plating of microbes were performed using nutrient agar and positive control was used of commercial antibiotic named ampicillin. Zone of inhibition was measured for each extracts of different tulsi species against the two bacterial strains. For disc diffusion Whatmann Filter paper 42 discs was used. For preparation of the extracts, 10 g of *Ocimum* leaves respectively was crushed in 200 ml of the solvents respectively and left for drying at 40 degrees C for the solvent extracts for 24 hours in incubator. The powders thus obtained from respective

conditions were dissolved in respective solvents to obtain variable concentrations to be used for ZOI studies. All experiments were carried out in triplicates, and with regard to the initial studies of ZOI done with all the three *Ocimum* studies, further ZOI studies was carried out with three different species of Tulsi.

RESULT AND DISCUSSIONS

1.Well Diffusion Method:

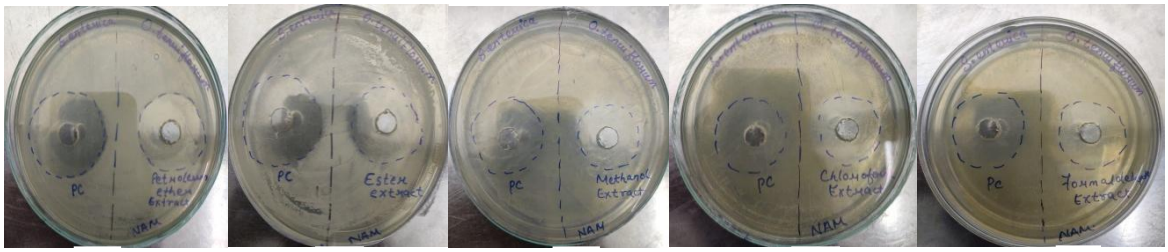
1.1*Ocimum tenuiflorum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum tenuiflorum*** using agar well-diffusion method against ***Salmonella enterica***.

Table.1.1:- Antibacterial activity at different extracts of *Ocimum tenuiflorum* concentration of against *Salmonella enterica*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	28 mm	PC	28 mm	PC	28 mm	PC	26 mm	PC	27 mm
60	25mm	60	25 mm	60	14mm	60	13mm	60	17 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 1.1:- Antibacterial activity of different extracts of *Ocimum tenuiflorum* against *Salmonella enterica*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum tenuiflorum*** using agar well-diffusion method against ***Salmonella enterica.***

Methanol extract @ 80 mg of *Ocimum tenuiflorum* showed a maximum zone of inhibition of 20.5 mm against *Alcaligens faecalis* whereas the minimum was recorded in formaldehyde extract @ 80 mg concentration.

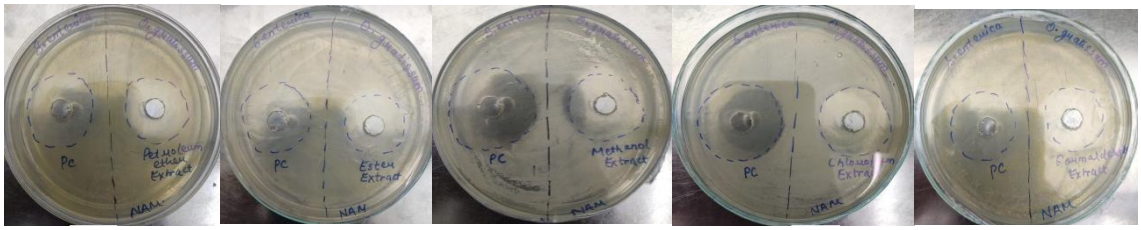
1.2 *Ocimum gratissum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum gratissum*** using agar well-diffusion method against ***Salmonella enterica***.

Table1.2 :- Antibacterial activity at different extracts of *Ocimum gratissum* concentration of against *Salmonella enterica.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	26 mm	PC	27 mm	PC	28 mm	PC	27 mm	PC	25 mm
170	14 mm	170	14.5 mm	170	16.3 mm	170	16.5mm	170	13.5 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 1.2 :- Antibacterial activity of different extracts of *Ocimumgratissum*against *Salmonella enterica*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum gratissum*** using agar well-diffusion method against ***Salmonella enterica.***

Chloroform extract of this species showed maximum ZOI whereas the minimum was recorded in 13.5 mm with formaldehyde extract.

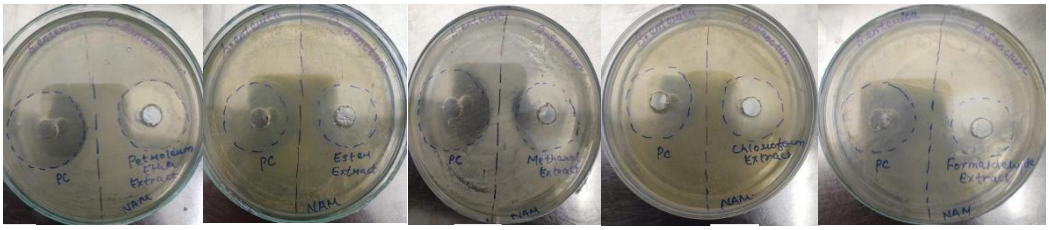
1.3 ***Ocimum sanctum***

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum sanctum*** using agar well-diffusion method against ***Salmonella enterica***.

Table1.3 :- Antibacterial activity at different extracts of *Ocimum sanctum* concentration of against *Salmonella enterica.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	25 mm	PC	24 mm	PC	24 mm	PC	24 mm	PC	25 mm
80	17mm	80	13mm	80	16mm	80	14 mm	80	12.5 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 1.3 :- Antibacterial activity of different extracts of *Ocimumsanctum*against *Salmonella enterica*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum sanctum*** using agar well-diffusion method against ***Salmonella enterica.***

Petroleum ether extract of this species showed maximum ZOI of 17 mm whereas the minimum was recorded in 12 mm with formaldehyde extract.

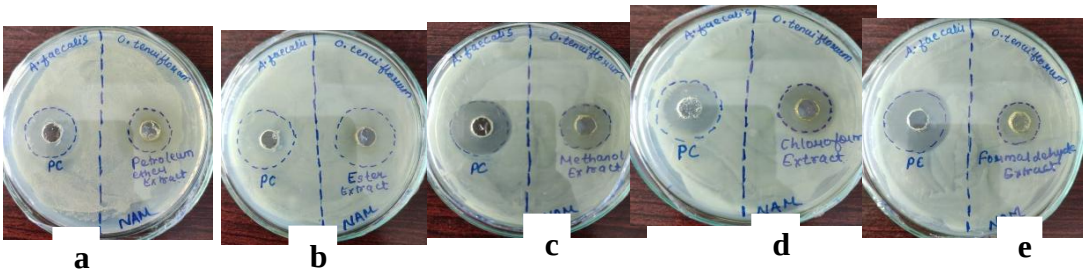
1.4 *Ocimum tenuiflorum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum tenuiflorum*** using agar well-diffusion method against ***Alcaligenes faecalis***.

Table1.4:- Antibacterial activity at different extracts of *Ocimum tenuiflorum* concentration of against *Alcaligenes faecalis.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	28 mm	PC	27 mm	PC	26 mm	PC	28 mm	PC	26 mm
80	14.5mm	80	18.1mm	80	20.5mm	80	13.2 mm	80	13 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 1.4 :- Antibacterial activity of different extracts of *Ocimum tenuiflorum* against *Alcaligenes faecalis* (a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum tenuiflorum*** using agar well-diffusion method against ***Alcaligenes faecalis.***

Methanol extract of this species showed maximum ZOI of 20.5 mm whereas the minimum was recorded in 13 mm with formaldehyde extract.

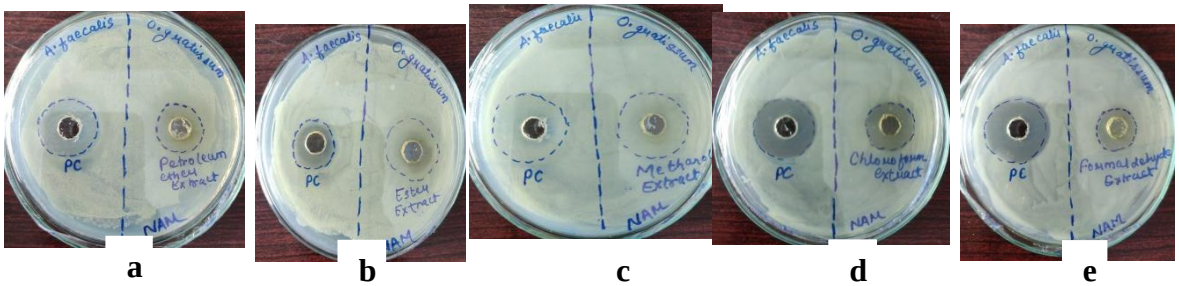
1.5 Ocimum gratissum

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum gratissum*** using agar well-diffusion method against ***Alcaligenes faecalis* .**

Table1.5 :- Antibacterial activity at different extracts of *Ocimum gratissum* concentration of against *Alcaligenes faecalis.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	28 mm	PC	28 mm	PC	26 mm	PC	27 mm	PC	27 mm
150	14.2mm	150	16.2mm	150	17.5mm	150	15mm	150	13.2 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 1.5 :- Antibacterial activity of different extracts of *Ocimum gratissum* against *Alcaligenes faecalis* (a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum gratissum*** using agar well-diffusion method against ***Alcaligenes faecalis.***

Methanol extract of this species showed maximum ZOI of 17.5 mm whereas the minimum was recorded in 13.2 mm with formaldehyde extract.

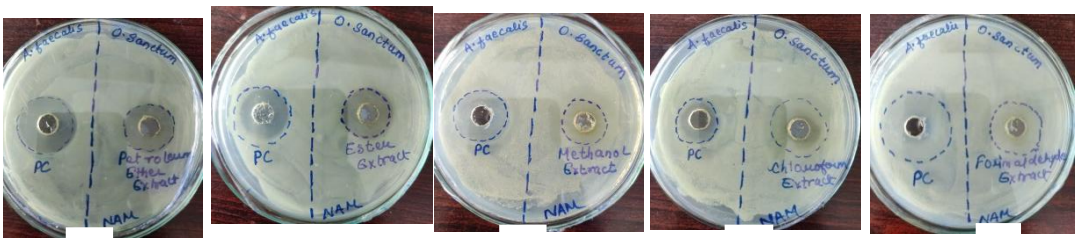
1.6 Ocimum sanctum

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum sanctum*** using agar well-diffusion method against ***Alcaligenes faecalis***.

Table1.6 :- Antibacterial activity at different extracts of *Ocimum sanctum* concentration of against *Alcaligenes faecalis.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	26 mm	PC	25 mm	PC	24 mm	PC	24 mm	PC	26 mm
100	15mm	100	17.5mm	100	13mm	100	17.5mm	100	16.5 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 1.6 :- Antibacterial activity of different extracts of *Ocimum sanctum* against *Alcaligenes faecalis* (a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum sanctum*** using agar well-diffusion method against ***Alcaligenes faecalis.***

Ester and Chloroform extract of this species showed maximum ZOI of 17.5 mmeach whereas the minimum was recorded in 15 mm with petroleum ether extract.

2. Disc Diffusion Method

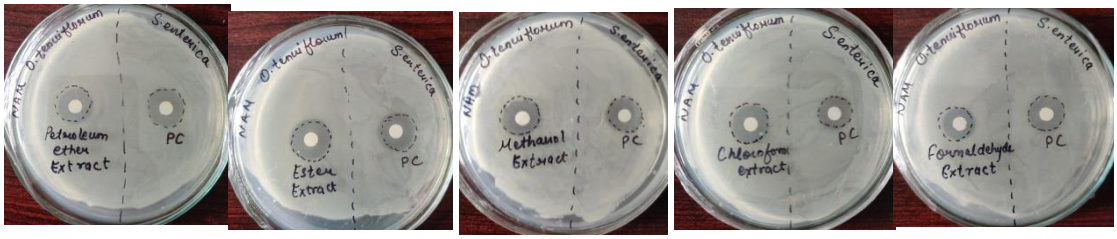
2.1 *Ocimum tenuiflorum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum tenuiflorum***usingdisc diffusion method against ***Salmonella enterica***.

Table2.1:- Antibacterial activity at different extracts of *Ocimum tenuiflorum* concentration of against *Salmonella enterica.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm
60	15 mm	60	20.2mm	60	20mm	60	21mm	60	14.8 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 2.1 :- Antibacterial activity of different extracts of *Ocimum tenuiflorum* against *Salmonella enterica*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum tenuiflorum*** using disc-diffusion method against ***Salmonella enterica.***

Chloroform extract of this species showed maximum ZOI of 21 mm whereas the minimum was recorded in 14.8 mm with formaldehyde extract.

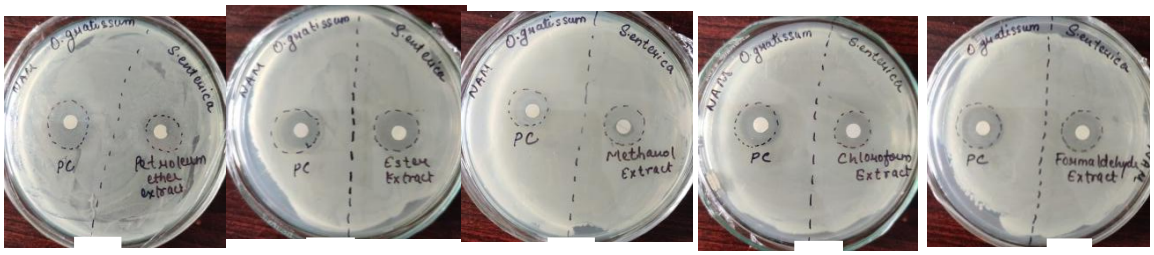
2.2 *Ocimum gratissum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum gratissum*** using disc-diffusion method against ***Salmonella enterica***.

Table2.2 :- Antibacterial activity at different extracts of *Ocimum gratissum* concentration of against *Salmonella enterica.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm
180	13.8mm	180	18mm	180	18mm	170	15mm	170	18 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 2.2 :- Antibacterial activity of different extracts of *Ocimum gratissum* against *Salmonella enterica*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum gratissum*** using disc-diffusion method against ***Salmonella enterica.***

Methanol and formaldehyde extract of this species showed maximum ZOI of 18 mm whereas the minimum was recorded in 13.8 mm with petroleum ether extract.

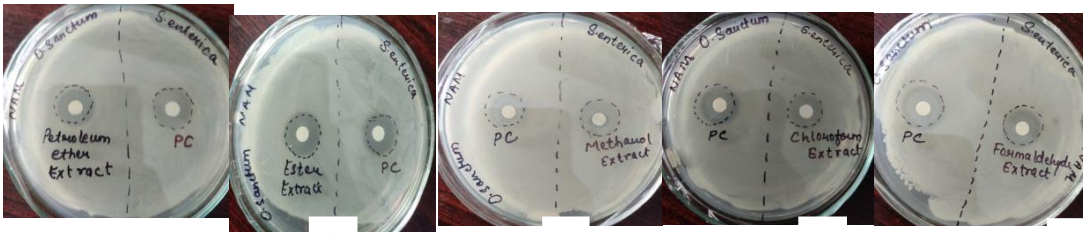
2.3 *Ocimum sanctum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum sanctum*** using disc-diffusion method against ***Salmonella enterica***.

Table2.3 :- Antibacterial activity at different extracts of *Ocimum sanctum* concentration of against *Salmonella enterica.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm
90	13.1 mm	90	16.5mm	90	16 mm	90	14mm	90	13.5 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 2.3 :- Antibacterial activity of different extracts of *Ocimum sanctum* against *Salmonella enterica*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum sanctum*** using disc-diffusion method against ***Salmonella enterica.***

Ester extract of this species showed maximum ZOI of 16.5 mm whereas the minimum was recorded in 13.1 mm with petroleum ether extract.

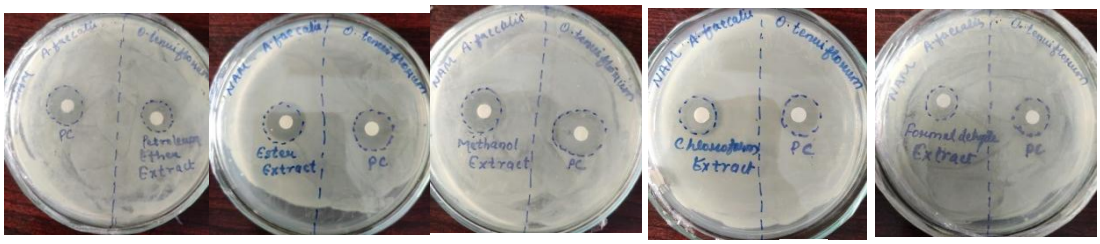
2.4 *Ocimum tenuiflorum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum tenuiflorum*** usingdisc-diffusion method against ***Alcaligenes faecalis***.

Table2.4 :- Antibacterial activity at different extracts of *Ocimum tenuiflorum* concentration of against *Alcaligenes faecalis.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	20 mm	PC	20mm	PC	20 mm	PC	20 mm	PC	20 mm
70	13 mm	70	18mm	70	20 mm	70	13mm	70	12.8 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 2.4 :- Antibacterial activity of different extracts of *Ocimum tenuiflorum* against *Alcaligenes faecalis*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum tenuiflorum*** using disc-diffusion method against ***Alcaligenes faecalis.***

Methanol extract of this species showed maximum ZOI of 20 mm whereas the minimum was recorded in 12.8 mm with formaldehyde extract.

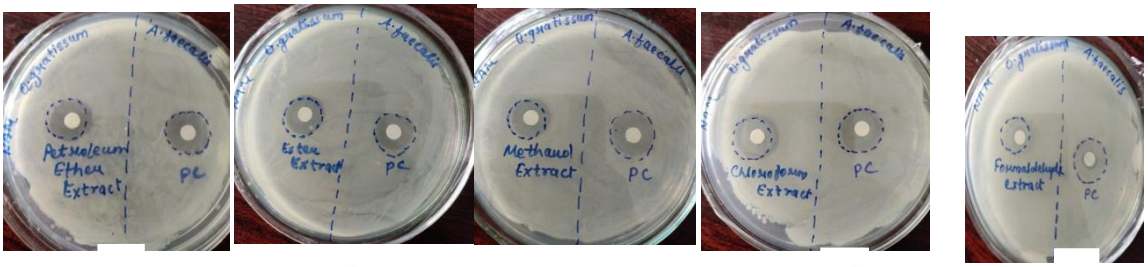
2.5 *Ocimum gratissum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum gratissum*** usingdisc-diffusion method against ***Alcaligenes faecalis***.

Table2.5 :- Antibacterial activity at different extracts of *Ocimum gratissum* concentration of against *Alcaligenes faecalis.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm
150	18 mm	150	17mm	150	17.2mm	150	18 mm	150	13 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 2.5 :- Antibacterial activity of different extracts of *Ocimum gratissum* against *Alcaligenes faecalis*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol ,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum gratissum*** using disc-diffusion method against ***Alcaligenes faecalis.***

Petroleum ether and chloroform extract of this species showed maximum ZOI of 18.0 mm whereas the minimum was recorded in 13 mm with formaldehyde extract.

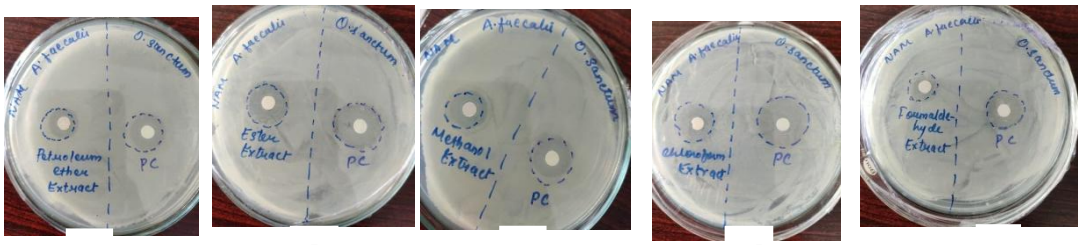
2.6 *Ocimum sanctum*

The results of in-vitro antimicrobial activity of the Chloroform, Methanol, ester, Formaldehyde and Petroleum ether extracts of ***Ocimum sanctum*** usingdisc-diffusion method against ***Alcaligenes faecalis***.

Table2.6 :- Antibacterial activity at different extracts of *Ocimum sanctum* concentration of against *Alcaligenes faecalis.*

Petroleum ether extract		**Ester extract**		**Methanol extract**		**Chloroform extract**		**Formaldehyde extract**	
Conc. (mg)	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**	**Conc. (mg)**	**ZOI (mm)**
PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm	PC	20 mm
100	16mm	100	18.2mm	100	17mm	110	17.5mm	100	12.5 mm

PC- Positive control (Ampicillin), ZOI- zone of inhibition



a **b** **c** **d** **e**

Fig 2.6 :- Antibacterial activity of different extracts of *Ocimum sanctum* against *Alcaligenes faecalis*(a) Petroleum ether extract, (b) Ester extract, (c) Methanol extract, (d) Chloroform extract and (e) Formaldehyde extract.

The results of in-vitro antimicrobial activity of the Chloroform, Methanol,ester, formaldehyde and Petroleum ether extracts of leaves of ***Ocimum sanctum*** using disc-diffusion method against ***Alcaligenes faecalis.***

Ester extract of this species showed maximum ZOI of 18.2 mm whereas the minimum was recorded in 12.5 mm with formaldehyde extract.

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