Antimicrobial activity of *Ocimum canum* Sims. (Pin-sein) (ပင်စိမ်း) on some bacteria and its chemical constituents

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## INTRODUCTION

- Herbal medicines the basis of health care throughout the world since the earliest days of mankind, are still widely used
- According to World Health Organization, medicinal plants the best source to obtain a variety of drugs

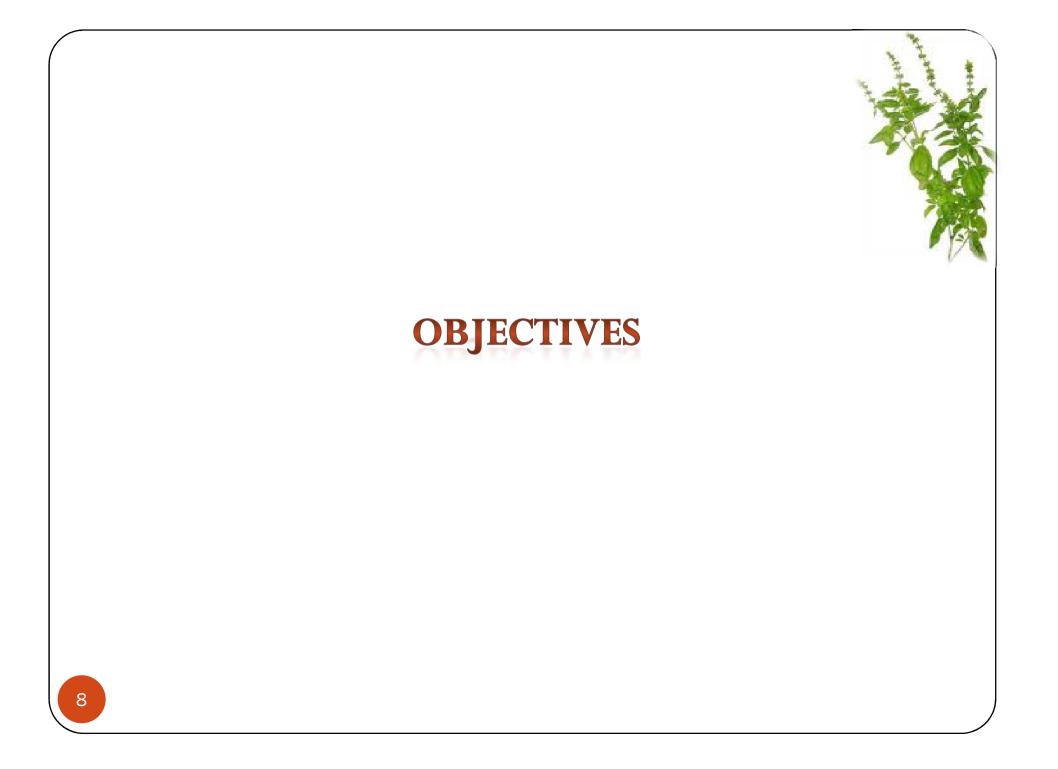
- Medicinal plants widely used in various ailments, because of their safety besides being economical, effective and their easy availability
- Apporximately 80% of the people in the world's developing countries rely on the traditional medicine for their primary health care needs

- The frequency of life-threatening infections an important cause of morbidity and mortality in developing countries (Ara *et al.*, 2009)
- Antibiotic resistance among bacterial pathogens is also increased in the hospital environment
- Antibiotics associated with adverse effects on the host
  - (Cordell, 2000)

- Therefore, new drugs needed to discover for infectious diseases
- Ocimum canum Sims.
  - ➤a characteristic odor and sharp taste
  - ≻used in curries
  - ➤easily grown in everywhere
- leaves and flowering tops perceived as carminative, stomachic

and antispasmodic in folk medicine (Sajjadi, 2006)

- the constituents of *Ocimum* species antibacterial, antifungal and antioxidant activities
- But no previous study on the antimicrobial activity of essential oil and extracts of *Ocimum canum* Sims. in Myanmar
- Ocimum canum Sims. (Pin-Sein) was chosen as medicinal plant to be studied



- To determine the phytochemical constituents of *Ocimum canum* Sims.
- To determine the *in-vitro* antimicrobial activity of essential oil and extracts on *Staphylococcus aureus*, *Klebsiella* species and *Proteus* species



## **MATERIALS AND METHODS**

#### **Materials**



## Collection of plant materials

The plant materials used in this study was collected from cultivated sources in Htauk Kyant, Mingaladon Township, Yangon, during the period from January to August, 2013

## Materials used for extraction of essential oil and different extracts from *Ocimum canum* Sims. leaves

Analytical Balance, Jacket coil condenser, Heating Mantle, Distillation apparatus, Separating funnel, Percolator, Evaporating basin, Glass wares, Vaccum rotary evaporator, Water-bath, Dessicator



### Chemicals

Acetic acid, Chloroform, Ethanol (50%),

Ethyl acetate, Ferric chloride, Hydrochloric acid, Sodium bicarbonate, Petroleum ether (60-90°C),

5% Potassium hydroxide, Sodium hydroxide

Materials used for determination of antimicrobial activity of Ocimum canum Sims. leaves Bacteria - control strains and clinical isolates of *Staphylococcus* aureus, Proteus species and Klebsiella species Media - Mueller Hinton agar (BBL, USA), Nutrient broth (Oxoid, England)

Discs for antimicrobial sensitivity tests

Antibiotic - Levofloxacin 5  $\mu$ g, Plain disc – 8mm diameter

#### **Methods**

Identification of plant



The plant was identified at Department of Botany, University of Yangon

• **Extraction of essential oil** from *Ocimum canum* Sims. by

using hydrodistillation method (British Pharmacopoeia 1988)

• Preparation of plant extracts for antimicrobial activity of crude extracts by using percolation method (British Pharmacopoeia 2007)

Phytochemical analysis of Ocimum canum Sims. leaves
 Qualitative analysis for the active constituents - the

 quality control methods according to WHO (1998)



#### Determination of antimicrobial susceptibility

Screening of antimicrobial activity was carried out by agar disc diffusion technique according to modified Kirby and Bauer method (WHO, 2003)

### Antimicrobial susceptibility testing

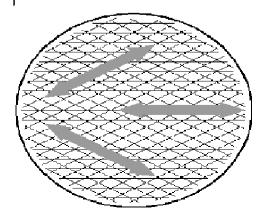


A sterile cotton swab

 $\succ$  dipped into the bacteria suspension

➤ streaked on the prepared media plate thoroughly in three directions (turning the plate by 60° after each

streak)



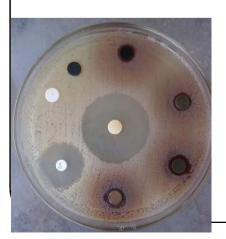


#### The discs

- impregnated with different extracts and essential oil
- placed on the medium approximately 15 mm form
   the edge of the plate (sterile needle tip)
- impregnate with solvent (negative control)
- levofloxacin disc (antibiotic control/ positive control)



➢ incubated in the incubator at 35°C
➢ the zone diameter (including 8 mm disc)
➢ complete inhibition of growth as determined by the naked eye is recorded as "sensitive"





## FINDINGS AND DISCUSSIONS

• Identification of plant

The plant was identified as *Ocimum canum* Sims. belonging to the family Lamiaceae (Labiatae)

• Yield percentage of essential oil of *Ocimum canum* Sims. leaves

Weight %	Volume %	Yield %	Yield %
		(w/w)	(v/w)
0.52	0.58	0.52	0.58

### Yield percentage of different extracts of Ocimum canum Sims. leaves

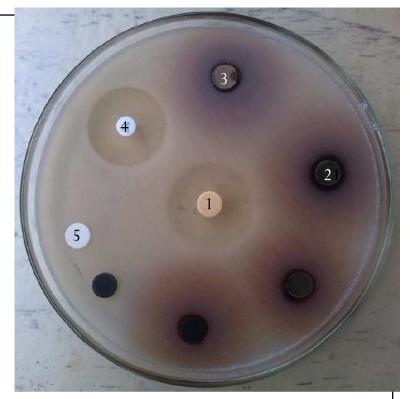
Solvent	Weight (g)	Yield (%)	
Distilled water	100 g	15.15	
50% Ethanol	100 g	19.859	

## Phytochemical investigations

No	Type of compound	Results
1.	Alkaloids	-
2.	Carbohydrates	-
3.	Glycosides	+
4.	Phenolic compounds	+
5.	<b>α-</b> amino acid	+
6.	Saponins	+
7.	Starch	-
8.	Tannins	-
9.	Flavonoid	+
10.	Steroids	+
11.	Reducing sugars	-
12.	Terpenoids	+
13.	Cyanogenetic glycoside	-

Antimicrobial activity of essential oil and									
different extracts of Ocimum canum Sims. leaves									
Test organisms		Diameter of inhibition zone (mm) of essential oil and different crude extracts							
		of Ocimum canum Sims. leaves							
		Essential oil	EtOH (50%)	Aqueous	LV (5µg)				
	C	50	21	13	32				
Staphylococcus aureus	CI	27	11	9	26				
	C	23	20	10	39				
<i>Proteus</i> species	CI	16	9	NA	34				
	C	20	12	10	28				
<i>Klebsiella</i> species	CI	14	NA	NA	24				
C=control, CI=clinical isolate, NA=no activity, LV=levofloxacin									



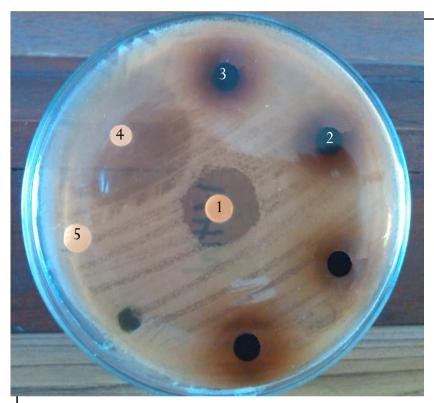


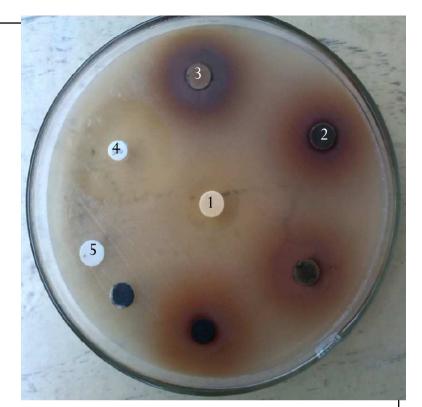
#### (a) Control strain

(b) Clinical isolate

## Effect of essential oil and different curde extracts of *Ocimum canum* Sims. on *Staphylococcus aureus*

 $1 = essential oil 20 \ \mu l/disc$ 2 = 50% ethanol extract 100 mg/disc $3 = Aqueous extract 120 \ mg/disc$  $4 = Levofloxacin 5 \ \mu g/disc (control)$ 5 = 70% ethanol 50 \ \mu l/disc (solvent control)





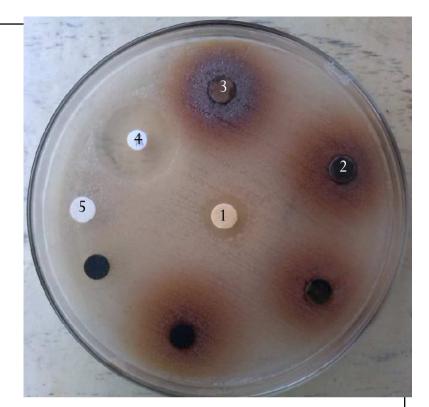
(a) Control strain

(b) Clinical isolate

# Effect of essential oil and different curde extracts of *Ocimum canum* Sims. on *Proteus* species

 $1 = essential oil 20 \ \mu l/disc$ 2 = 50% ethanol extract 100 mg/disc $3 = Aqueous extract 120 \ mg/disc$  $4 = Levofloxacin 5 \ \mu g/disc (control)$ 5 = 70% ethanol 50 \ \mu l/disc (solvent control)





#### (a) Control strain

(b) Clinical isolate

## Effect of essential oil and different curde extracts of *Ocimum canum* Sims. on *Klebsiella* species

 $1 = essential oil 20 \ \mu l/disc$ 2 = 50% ethanol extract 100 mg/disc $3 = Aqueous extract 120 \ mg/disc$  $4 = Levofloxacin 5 \ \mu g/disc (control)$ 5 = 70% ethanol 50 \ \mu l/disc (solvent control)

#### Discussions

- Many plant species have been used by the people as a traditional medicine, including as treatments for infectious diseases
- In this present work,
  - essential oil,
  - ethanolic (50%) and
  - aqueous extracts

antibacterial activity on

Staphylococcus aureus,

Proteus species and

Klebsiella species

- To summarize, essential oil of Ocimum canum Sims.
   leaves showed most potent activity Staphylococcus aureus
- In the investigation of chemical constituents, Ocimum canum Sims. leaves powder possess glycosides, phenolic compounds, flavoniod, terpenoids, steroids, saponins and α-amino acid
- So, the essential oil of *Ocimum canum* Sims. leaves can be used in the development of pharmaceutical formulation



## Suggestions

To study the **anti-diabetic activity and antidiarrhoea activity** of *Ocimum canum* Sims. leaves extract on experimental animals

#### References

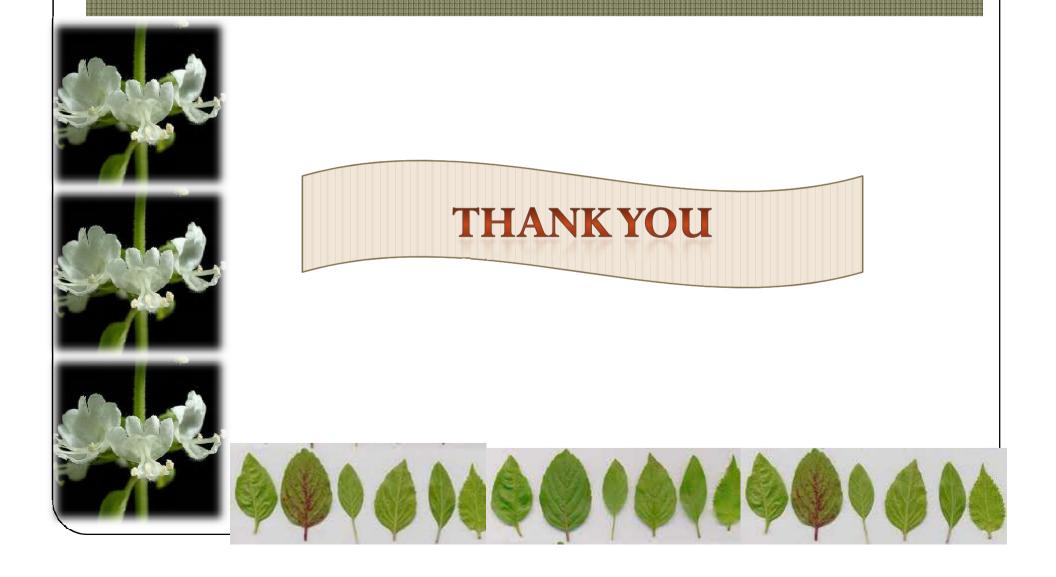
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## Herbal is a word of high value now



- Thousands of medicinal plants species found in different parts of the Myanmar used from the ancient time
- The government of Myanmar is giving impetus
  - ➤ to develop traditional medicine systematically
  - ➢ to reach international standards
  - ➤ to manufacture potent and efficacious traditional medicine based on scientific evidences and practices

- Therefore, new drugs needed to discover for infectious diseases
- Plants contain numerous biologically active compounds, many of which have been shown to have antimicrobial properties
- In order to promote the use of medicinal plants (antimicrobial compounds) - investigate their active constituents and activity and thus validate their use

#### Laboratory glasswares and other apparatus

Petridishes (Pyrex), Universal containers, Swab sticks,

Micropipette (5-50 $\mu$ L) & pipette tips

Chemical

 $0.5 \% BaSO_4$  solution

#### Standard laboratory equipments

Autoclave (GallenKamp), Incubator (GallenKamp), Electronic

balance, Hot air oven (GallenKamp)

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Extraction of essential oil from Ocimum canum Sims.
           by using hydrodistillation method
                  British Pharmacopoeia (1988)
               air-dried leaves (dried for 2 days)
                              ↓ cut into pieces
               100g of leaves +500mL of distilled water
                placed in a round-bottomed flask
                    placed in a heating mantle
                               heated to boils for 3-4 hours
               distillate (a mixture of oil and water)
                                passed through the condenser, collected in
                               the receiver
                                separated by a glass
                                separating funnel
                          essential oil
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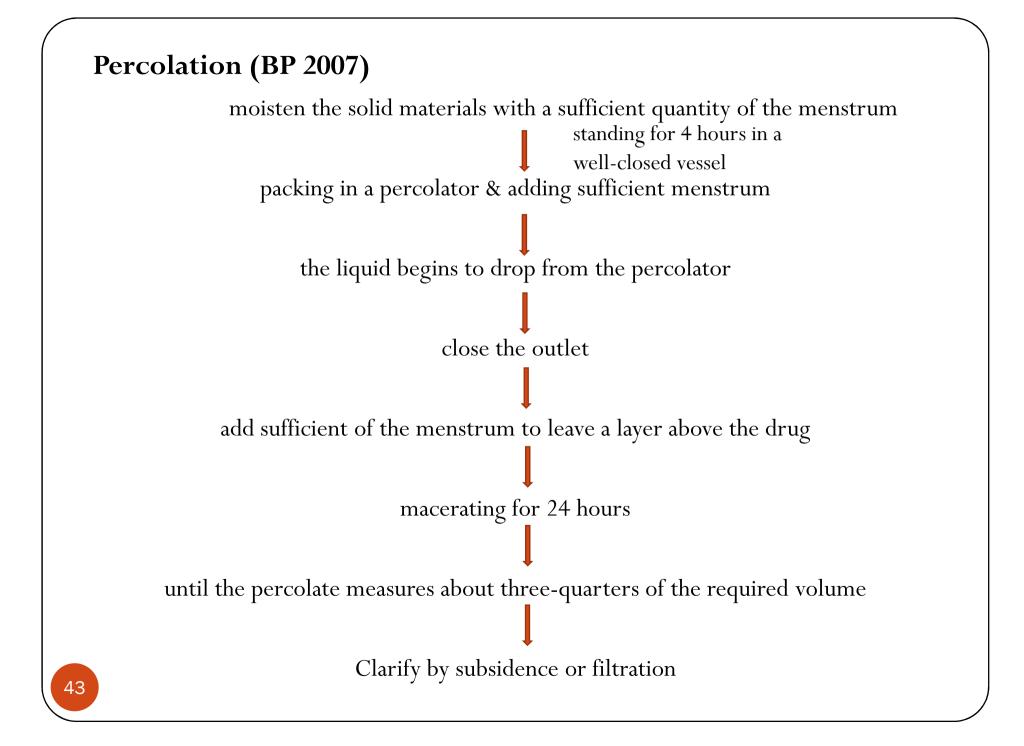
Hydrodistillation apparatus for extraction of essential oil

# Preparation of plant extracts for antimicrobial activity of crude extracts

(British Pharmacopoeia 2007)

- the leaves air-dried for one week
- reduced into coarse powder by using clean mortar and pestle
- powdered material (100g) percolated with 50% ethanol
   for a week by using the percolator

- For aqueous extract, 50 g of powdered leaves extracted with 250 mL distilled water by reflux apparatus
- all liquid extracts were evaporated separately by using a rotatory evaporator and water-bath to give a dry mass

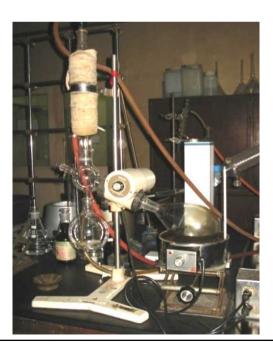


Reflux apparatus for aqueous extract





Percolator for extraction of plant materials



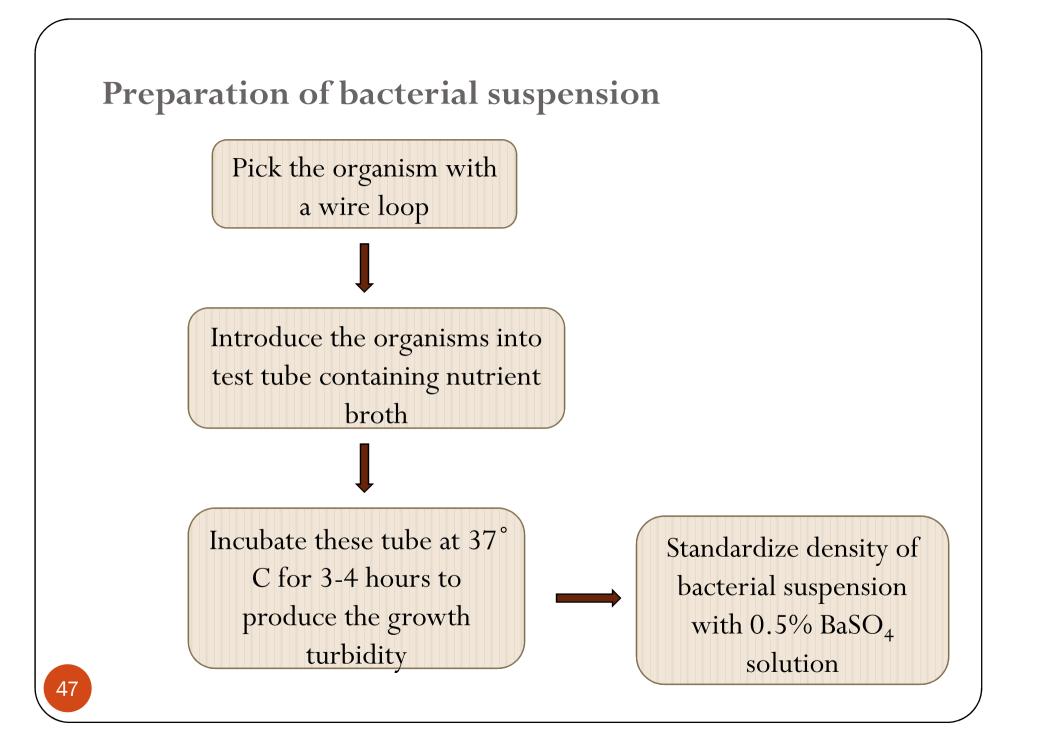
Rotary evaporator for solvent evaporation

## Preparation of discs impregnated with essential oil and different extracts

- For 50% EtOH,
  - > 1g of extract into 1 ml of solvent (1 g/mL) (stock solution)
  - $\blacktriangleright$  discs impregnated with 100 µL (100 mg/disc)
- For aqueous extract,
  - > 1.2g extract into 1 ml of solvent (1 g/mL) (stock solution)
  - $\blacktriangleright$  discs impregnated with 100 µL (120 mg/disc)
- For essential oil,
  - > 20  $\mu$ L of oil added to discs (20  $\mu$ L/disc)

#### **Preparation of medium**

- Muller Hinton agar prepared according to manufacture's recommendations, autoclaved
- ▶ 20 mL of the media poured into 115 mm petridishes, allowed to set at room temperature (freshly prepare)
- ➤When the agar had solidified, the plates were dried (upright position) in the incubator





### **CONCLUSIONS AND SUGGESTIONS**

48

#### Conclusions

- The phytochemical data glycosides, phenolic compounds, flavonoid, terpenoids, steroids, saponins and  $\alpha$ -amino acid
- essential oil the broad spectrum of action against *Staphylococcus aureus*, *Proteus* species and *Klebsiella* species

#### Suggestions

To study the anti-diabetic activity and anti-diarrhoea activity of

Ocimum canum Sims. leaves extract on experimental animals

