ANTIBACTERIAL ACTIVITY OF TUBEROUS ROOT EXTRACTS OF *STEMONA COCHINCHINENSIS* GAGNEP. (ဂုံသမျှား)

Theingi Swe[1], Khin Htet Zin[2], Kyi Kyi Oo[3], Yadanar[2], Soe Myint Aye[4], Ni Ni Zaw[5], Myintzu Tin Oung[6] & Theim Kyaw[2]

1. Traditional Medicine Clinic, Katha Township, Sagaing Region
2. University of Traditional Medicine, Mandalay
3. Department of Traditional Medicine, Naypyitaw
4. Department of Higher Education, Napyitaw
5. Department of Microbiology, Mandalay General Hospital
6. Department of Medical Research (Pyin Oo Lwin Branch)

Introduction

- WHO estimated that nearly 80% of the population of developing countries rely on traditional medicine, predominantly originated from plants for their primary health.
- Nearly 25% of modern medicine are derived from plants first used in traditional medicine.
- Herbal drugs have been increasingly popular in their uses of antimicrobial activity.
- Microorganisms frequently associated with wound infections are *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*

- In one study in Laos, percentage inhibition of crude ethanolic extracts of *S. cochinchinensis* Gagnep. used to treat different types of cough were 14.73% for *S.aureus*, 30.75% for *E.coli*, 52.98 % for *M.tuberculosis* H37Rv (Elkington *et al.*,2011).
- The present study is designed to study the antibacterial activity of aqueous, ethanolic and methanolic tuberous root extracts of *S.cochinchinensis* Gagnep.

General objective

- To study the antibacterial activity of aqueous, ethanolic and methanolic tuberous root extracts of *Stemona cochinchinensis* Gagnep.

Specific objectives

1. To assess the preliminary phytoconstituents of aqueous, ethanolic and methanolic tuberous root extracts of *Stemona cochinchinensis* Gagnep.
2. To determine the antibacterial activity of aqueous, ethanolic and methanolic tuberous root extracts of *Stemona cochinchinensis* Gagnep. on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*
3. To evaluate dose response relationships among three extracts on *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*
4. To compare the antibacterial activity of aqueous, ethanolic and methanolic tuberous root exracts of *Stemona cochinchinensis* Gagnep. on selected strains

Plant in natural habit

The whole plant

Flower

Fruit

leaves

Roots

Morphological characters of *S. cochinchinensis* Gagnep.

Methodology

Study Design Laboratory based experimental study

Study site

1. Department of Botany, University of Mandalay
2. Research Division, University of Traditional Medicine, Mandalay
3. Department of Microbiology, University of Medicine, Mandalay

Study Period From 1st August 2016 to 31st July 2017

Materials

1. Materials for Plant Extract

- Tuberous Roots of *S. cochinchinensis* Gagnep.
- Reflux extractor (No. 00MA14, Japan)
- Water bath (Model LD- 220, Japan)
- Vacuum rotary evaporator (BM 400, Japan)
- Freeze dryer (model FD-1, Japan)
- Beaker (500ml, 1000ml)
- Filter paper
- Round bottle
- Desiccator
- Electric balance

2. Materials for Collection of Samples and Isolation of Microorganisms

- Sterile petri dishes (RKI, Japan)
- Sterile cotton swab sticks
- Glass slides
- Cover slips
- Sterile test tubes (RKI, Japan)
- Bijou bottles
- Sprit lamp and inoculating wire loop, aluminium foil

3. Standard Laboratory Equipments for Isolation and Identification of Microorganisms

- Autoclave (Model YX-280A, Japan)
- Trimline Incubator (Y 7013996, London, England)
- Hot Air Oven (3/ 250 FC, Griffin)
- Microscope (G-302, Taiwan)
- Refrigerator
- Timer

4. Materials for Antimicrobial Susceptibility Test

- McFarland turbidity standard tube No.2
- Sterile normal saline
- Control bacteria strains (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922)
- Filter paper (Whattman No-3, England)
- Standard antibiotic discs; Cefotaxime 30µl (Himedia, India)
- Micropipette (1- 200 µl, Humapette)
- Vortex Mixer (VM 2000, Taiwan)
- Measuring flasks and cylinders (500ml, 1000ml)

5. Media for Isolation

- Nutrient agar (Himedia M001, India)
- Manitol salt agar (Himedia M118-500G, India)

6. Media and reagent for Identification

- Peptone water Medium (Himedia M028-500G, India)
- Gram's stain reagents

7. Media for Antimicrobial Susceptibility Test

- Muller-Hinton agar(Himedia M173-500G,India)
- Nutrient agar (Himedia M001, India)
- Nutrient broth

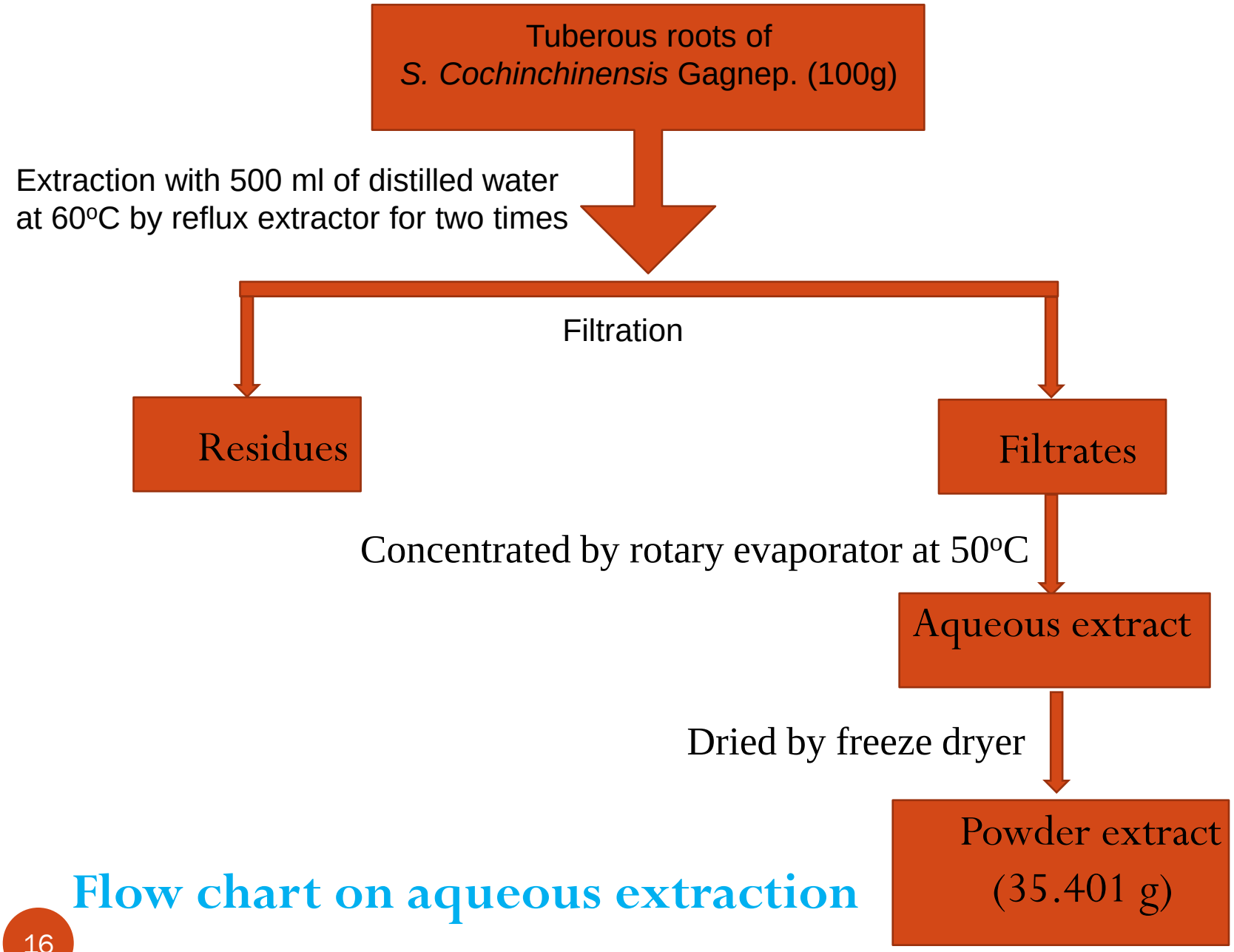
Methods

1. Plant Collection and Identification

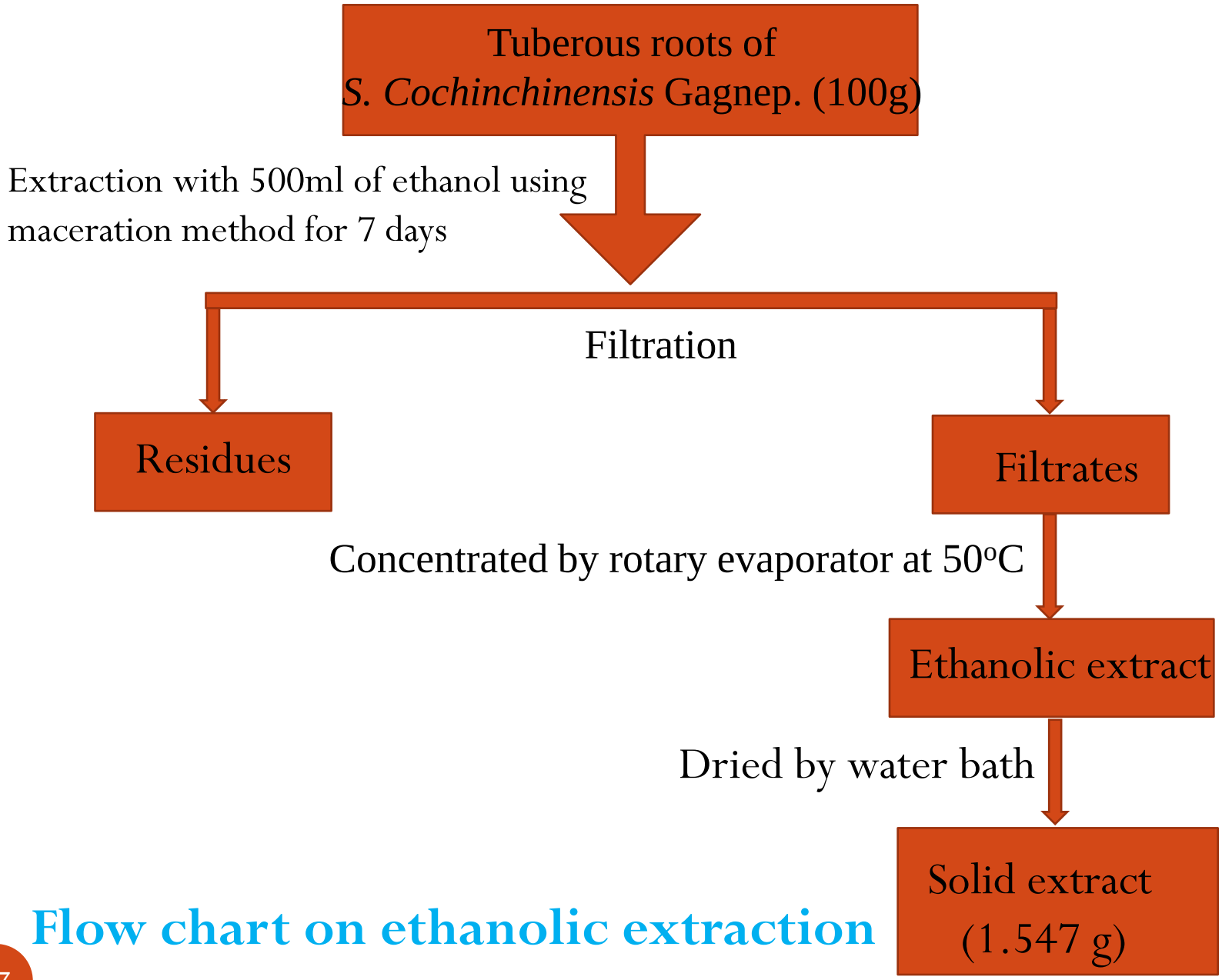
- The plant specimens were collected from Hteegyaint township, Sagaing Region and identified by the authorized Taxanomist from Department of Botany, University of Mandalay.
- The roots will be collected in November before flowering because of annual plant (AHPA, 2006).
- The samples were washed with water, chopped into small pieces, air dried at room temperature, weighed , pulverized by grinding and stored in air tight container.

2. Phytochemical Screening

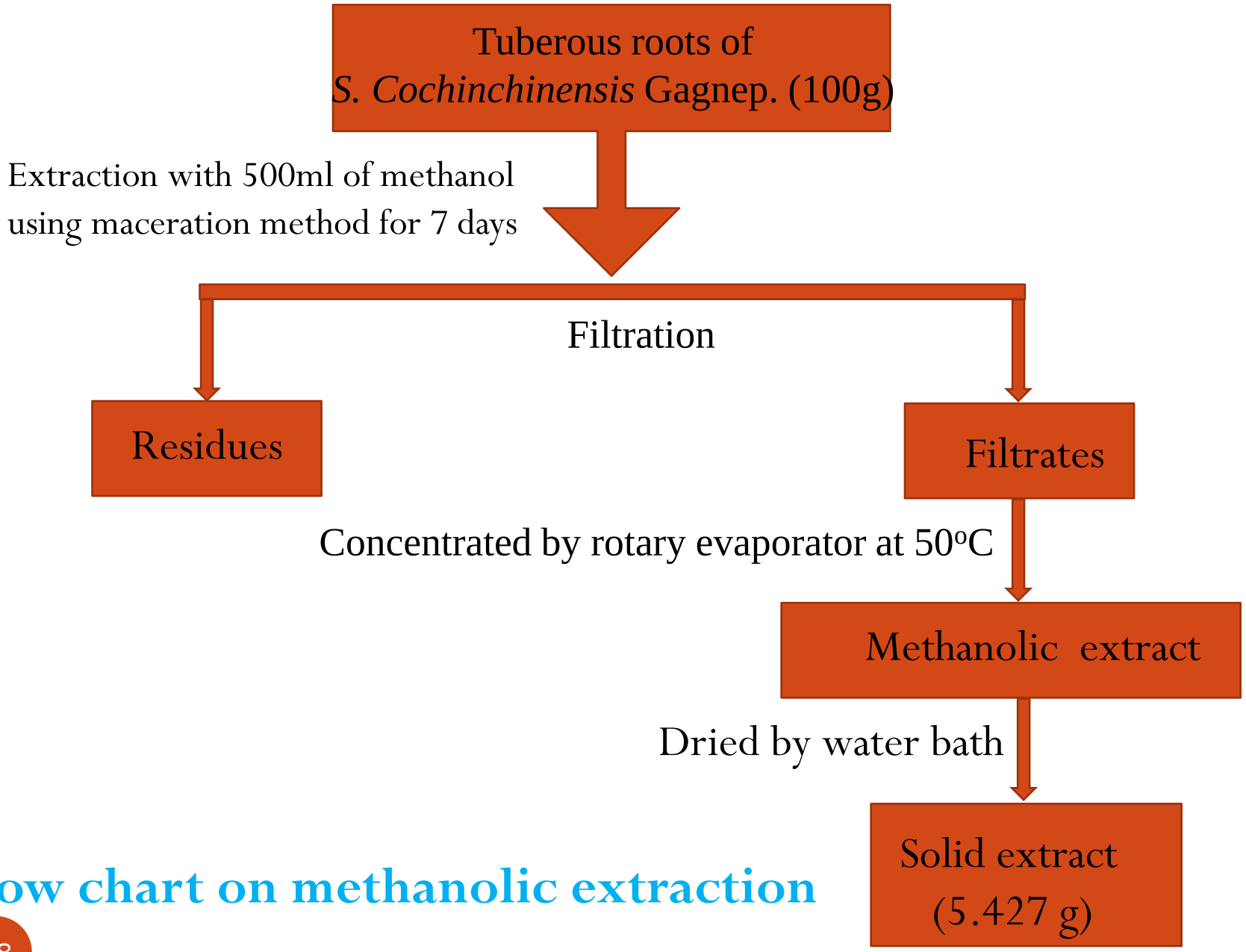
- Alkaloids
- Flavonoids
- Glycosides
- Phenolic Compounds
- Polyphenol
- Polysterols
- Saponins
- Reducing Sugar
- Amino Acid
- Carbohydrates
- Tannins
- Acid/Base/Neutral
- Cyanogenic Substance



Flow chart on aqueous extraction



Flow chart on ethanolic extraction



Flow chart on methanolic extraction

Determination of Antibacterial Activity

- Antibacterial activity was determined against three bacterial pathogens by agar dilution method and agar disc diffusion method.

(1) Bacterial Strains

- The test organisms (*Staphylococcus aureus* ATCC 25923, *Pseudomonus aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922) will be obtained from Upper Myanmar Public Health Laboratory, Mandalay.
- All the strains will be confirmed by cultural and biochemical characteristics and maintained in slants for further use at Department of Microbiology, University of Medicine, Mandalay.

(2) Culture Media

- Nutrient broth for making inoculums, appropriate medias and reagents for cultural and biochemical characteristics and Muller-Hinton agar for antimicrobial susceptibility will be used.

1. Agar dilution method

(1) Preparation of Agar Medium and Plant Extracts

- The Muller-Hinton agar medium will be prepared in large screwed – capped tubes or flasks.
- The agar will be allowed to cool in a water bath of 50 to 55 ˚C.
- The tuberous root extracts were diluted with methanol, 95% ethanol and distilled water for different serial dilutions(2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml and 10 mg/ml).
- Then, 1ml of different dilutions of each test sample was poured into 100mm petridishes and mixed with agar medium 19 ml respectively.

- The medium was shaken well and allowed to harden resulting for different concentrations of agar medium (100µg/ml, 150µg/ml, 200µg/ml, 250 µg/ml, 300 µg/ml, 350 µg/ml, 400 µg/ml, 450 µg/ml and 500 µg/ml).
- These agar media with different concentrations were duplicated to take mean value.

Table 1. Method of preparation of dilutions of antimicrobial agents for use in agar dilution susceptibility test

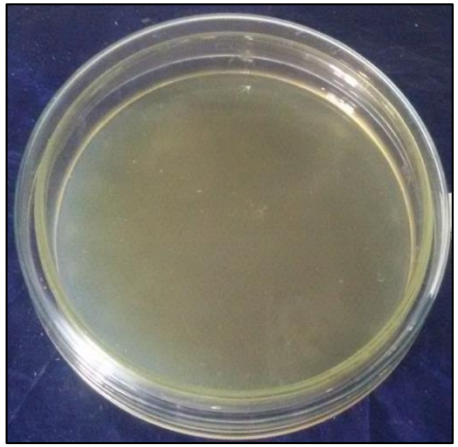
Antimicrobial concentration obtained before addition of 19ml agar(mg/ml)	Final concentration in medium after addition of 19ml agar(µg/ml)
10	500
9	450
8	400
7	350
6	300
5	250
4	200
3	150
2	100

(2) Preparation of Inoculums

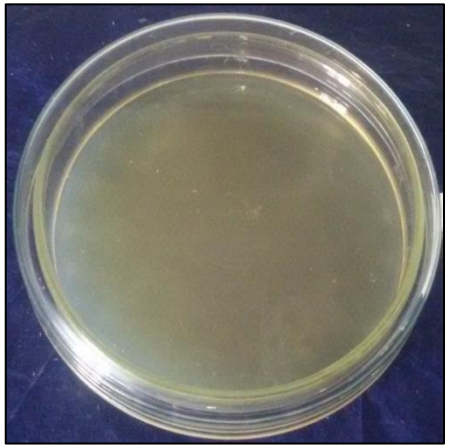
- A few colonies of the organism to be tested were picked from nutrient agar with a wire loop (Direct Colony Suspension Method) and inoculated into a test tube containing 2ml of nutrient broth and labeled.
- The tubes were incubated at 35ºC for three to four hours to produce the growth turbidity of 10^5organisms per ml.
- The turbidity of the resulting suspension was visually compared with 0.5 McFarland turbidity standards ($1\text{-}2x10^8$ CFU/ ml) .
- Then the test organisms were inoculated into peptone water.

(3) Control System

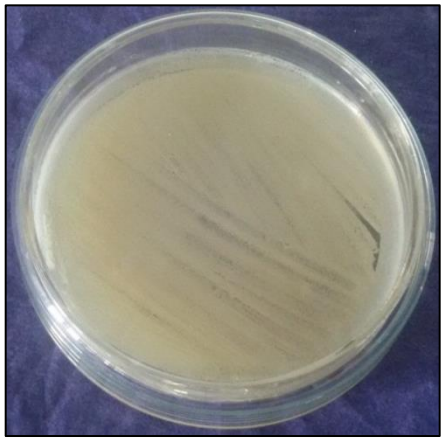
- Several control plates was prepared such as agar control plate, solvent control plate, organisms control plate, sterility control plate.
- Agar control plate was prepared by agar plate only.
- Solvent control plate was prepared by adding solvent into agar.
- Organisms control plate was prepared by inoculating tested organisms into agar plate without drugs and solvent.
- Sterility control plate was prepared by adding highest tested concentration of extract into agar.



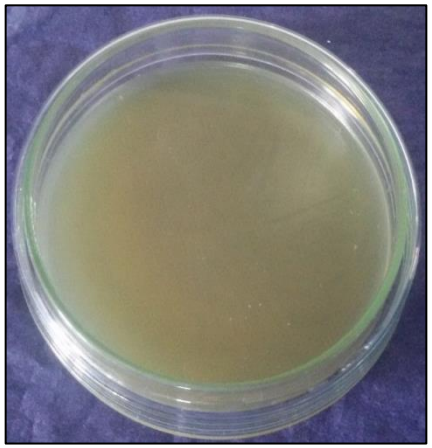
Sterile Muller-Hinton agar



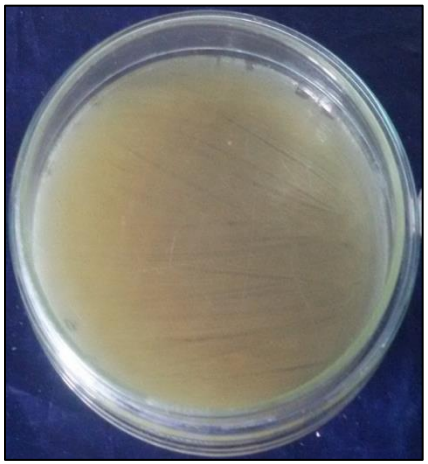
Sterile Muller-Hinton agar with distilled water



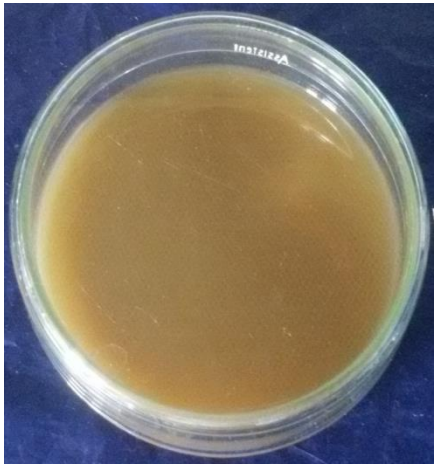
Growth of *S aureus*



Growth of *P aeruginosa*



Growth of *E coli*



500 µg/ml of aqueous extract

Control strains

(4) Agar dilution susceptibility test

- When the agar medium with different serial dilutions had solidified, the sterile cotton swab was dipped into the bacterial suspension and streaked by rotating onto the whole surface of the agar plates.
- Then all the plates were placed in upright position in the incubator with the lids tilted and then labeled.
- All plates were inverted and incubated for 24 hrs at 37^{o}C for 24-48 hours. After incubation, the control plates must be checked first to see if all organisms grew.
- The end point (MIC) was considered the plate in which there was no visible growth.

2. Disc diffusion method

(1) Plant Extracts Dilution

- The plant extracts will be diluted with distilled water, 95% ethanol, and methanol for different serial dilutions (50mg/ml, 100mg/ml, 150mg/ml, 200mg/ml and 250mg/ml).
- From each serial dilution of each extract, 20µl of test sample extracts was impregnated to each sterile disc.
- Thus the discs were completely saturated with the test samples resulting 1mg/disc, 2mg/disc, 3mg/disc, 4mg/disc and 5mg/disc respectively.

(2) Preparation of Agar Medium and discs

- The agar medium will prepared in large screwed – capped tubes or flasks capable of easily holding at least 25ml of fluid.
- The agar will be allowed to cool in a water bath of 50 to 55 ˚C and then be poured into each Petri dish, shakened well and allowed to harden.
- The discs which are 6mm in diameter, were punched from No.3 Whatman filter paper and sterilized by autoclaving followed by dry heat at 60^{o}C for 1hr.
- It was then impregnated with different concentrations of each extract and allowed to be dried in Pickstone oven.
- Cefotaxime (30µg) was used as standard antibiotic control disc.

(3) Preparation of Inoculums

- The inoculums will be prepared by making a direct broth suspension of isolated colonies selected from an 18 to 24 hour agar plate (Direct Colony Suspension Method).
- The turbidity of the resulting suspension will be visually compared with 0.5 McFarland turbidity standards (1-2x10^8 CFU/ml).

(4) Control System

- For each bacterial strain, standard antibiotic control disc, cefotaxime(30µg) was served as the positive antibacterial control and negative control were done using paper disc loaded with 20 µl of respective solvents.

(5) Antibacterial Susceptibility Testing

- After autoclaving the Muller Hinton agar, 25 ml of the media was poured into 100 mm petridishes and allowed to set at room temperature.
- When the agar had solidified, the plates were dried at 35ºC by placing them in upright position in the incubator with the lids tilted and then labeled.
- A sterile cotton swab was dipped into the bacterial suspension and streaked by rotating onto the whole surface of the agar plates.

- After the inoculum had dried at room temperatue, the prepared discs impregnated with plant extract, the control antibiotic disc; Cefotaxime (30µg) and solvent control disc were placed with a flamed forceps and gently pressed onto the surface of agar medium.
- After incubation in the incubator at 37 ºC for 24-48 hours, the diameter of the zone of inhibition surrounding bacterial growth were measured and expressed in (mm).
- The experiments were done in duplicate sets and the mean values were presented.

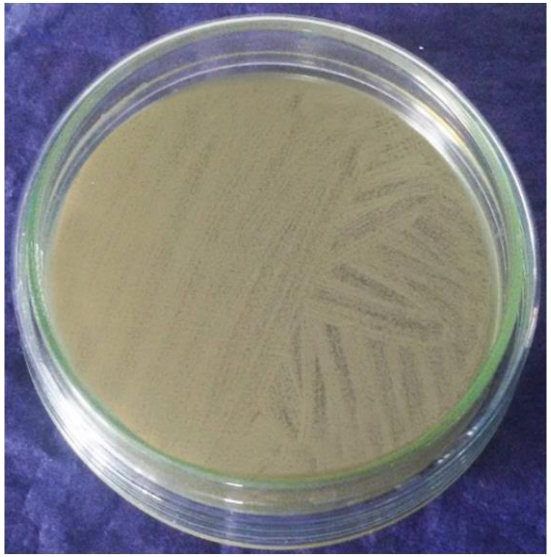
Findings

Phytochemical Analysis of three extracts of *S. cochinchinensis* Gagnep.

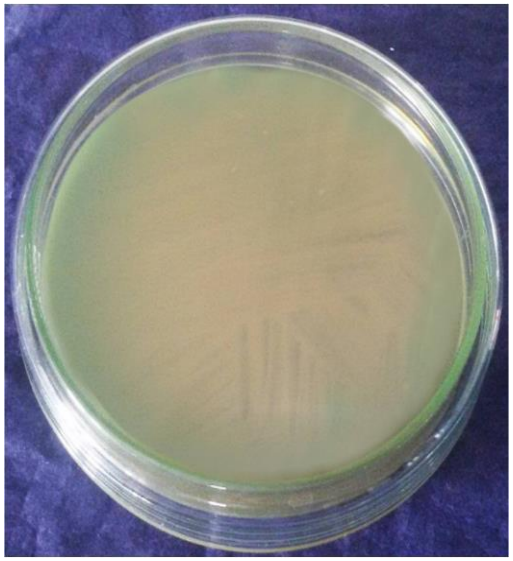
- The qualitative analysis indicated that the alkaloids, polyphenol, terpenoids, amino acid, carbohydrate and tannins are universally present in all three extracts but the glycosides, reducing sugar and cyanogenic substances are absent.
- The flavonoids were found in both ethanolic and methanolic extracts.
- Phenolic compound and saponin were present in methanolic extract.

Antibacterial activity of three extracts in agar dilution method

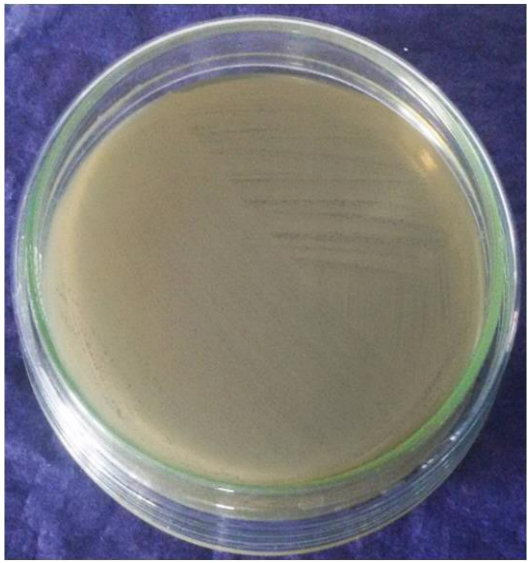
- The growth of all tested organisms was found in all concentrations of these extract ranging from 100µg/ml to 500µg/ml.
- MIC of the three extracts of tuberous root was not determined on *S. aureus*, *P. aeruginosa* and *E. coli* in the tested concentrations in this study.



Growth of *S aureus*

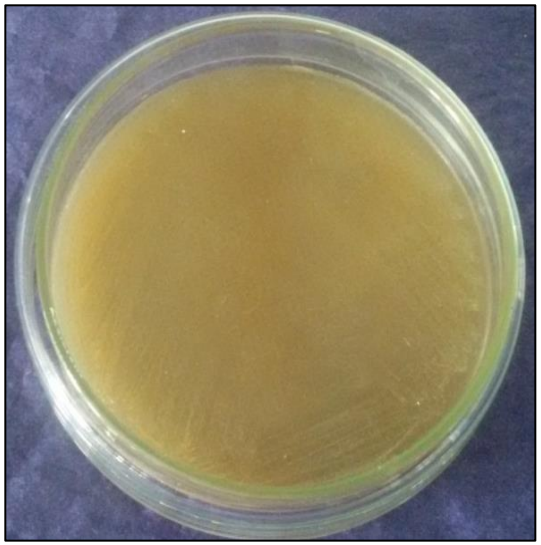


Growth of *P aeruginosa*

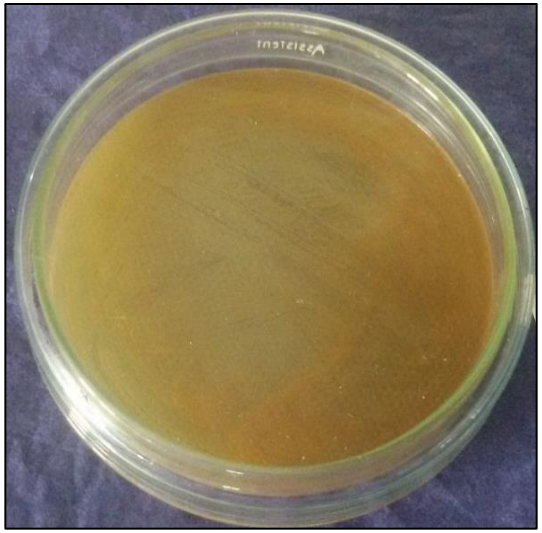


Growth of *E coli*

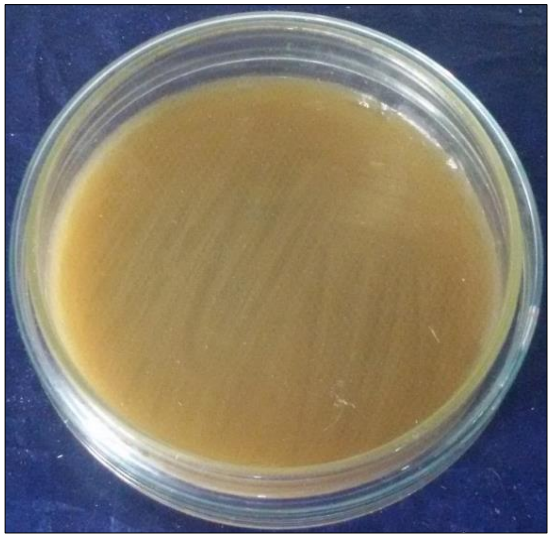
Figure1. Antibacterial activity of 500 µg/ml of aqueous extract in agar dilution method



Growth of *S aureus*

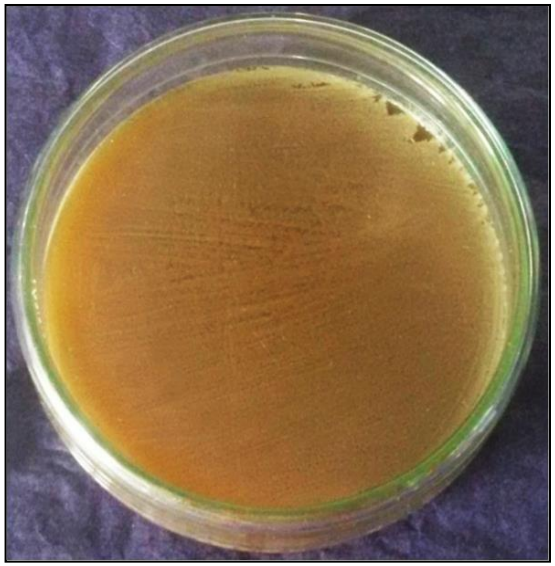


Growth of *P aeruginosa*

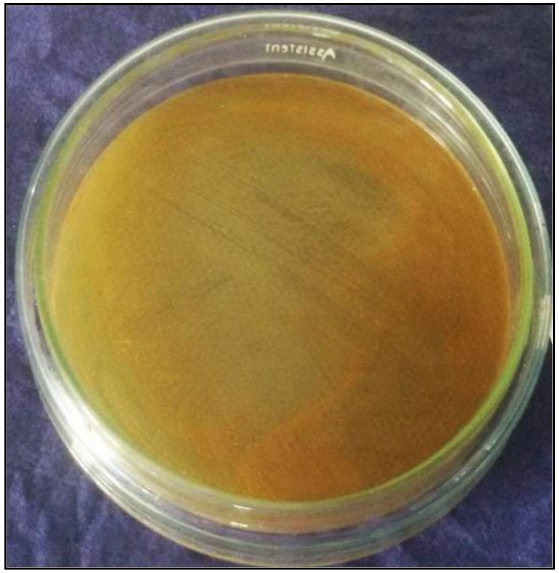


Growth of *E coli*

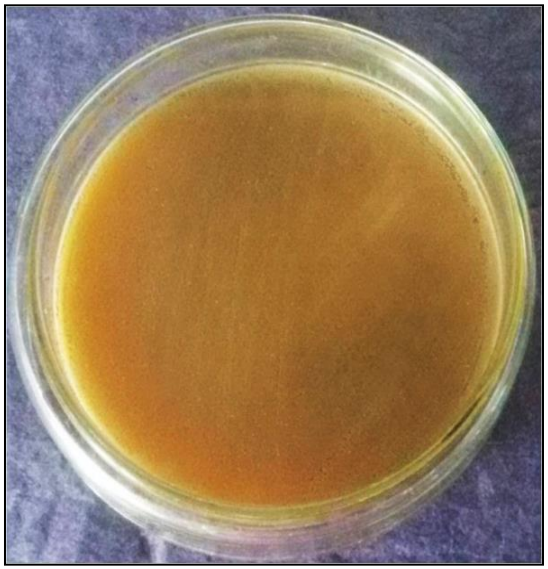
Figure2. Antibacterial activity of 500 μg/ml of ethanolic extract in agar dilution method



Growth of *S aureus*



Growth of *P aeruginosa*

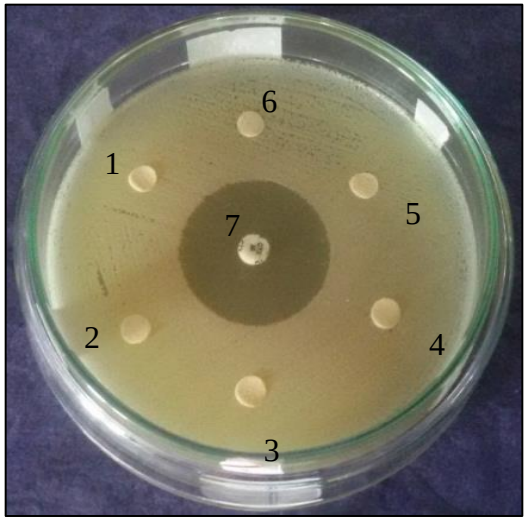


Growth of *E coli*

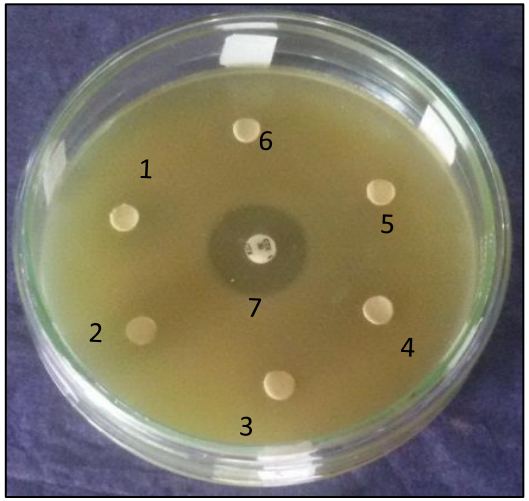
Figure 3. Antibacterial activity of 500 µg/ml of methanolic extract in agar dilution method

Antibacterial activity of aqueous extract in disc diffusion method

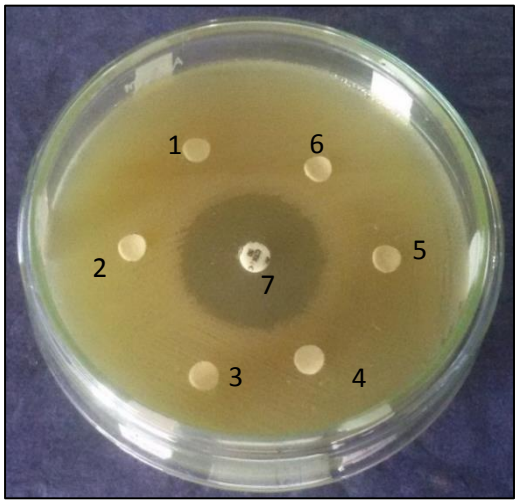
- The different concentrations of aqueous extract, solvent control disc with distilled water and cefotaxime (30 µg/disc) were tested on three control strains of bacteria.
- The inhibition zones were not found.
- The average zone of inhibition for the standard antibiotics disc, cefotaxime was 31 mm, 20 mm and 30 mm on *S. aureus*, *P. aeruginosa* and *E.coli* respectively.



S aureus



P aeruginosa



E coli

1 = 1 mg/disc concentration
2 = 2 mg/disc concentration
3 = 3 mg/disc concentration
4 = 4 mg/disc concentration
5 = 5 mg/disc concentration
6 = Control disc, Distilled water
7 = Cefotaxime (30 µg/disc)

Figure 4. Antibacterial activity of aqueous extract in disc diffusion method

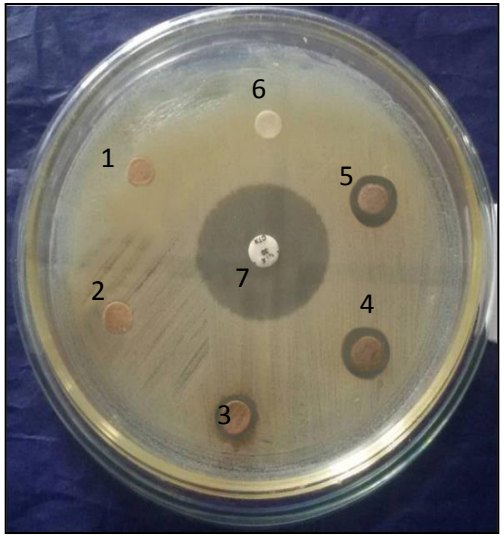
Table 2. Antibacterial Activity of Aqueous Extract of roots of *S. cochinchinensis* Gagnep. for Disc Diffusion Method.

Conc (mg/disc)	Zone of inhibition in mm		
	Staphylococcus aureus	*Pseudomonus aeruginosa*	*Escherichia coli*
1(mg/disc)	0 mm	0 mm	0 mm
2(mg/disc)	0 mm	0 mm	0 mm
3(mg/disc)	0 mm	0 mm	0 mm
4(mg/disc)	0 mm	0 mm	0 mm
5(mg/disc)	0 mm	0 mm	0 mm
Water	0 mm	0 mm	0 mm
Cefotaxime (30 µg/disc)	31 mm	20 mm	30 mm

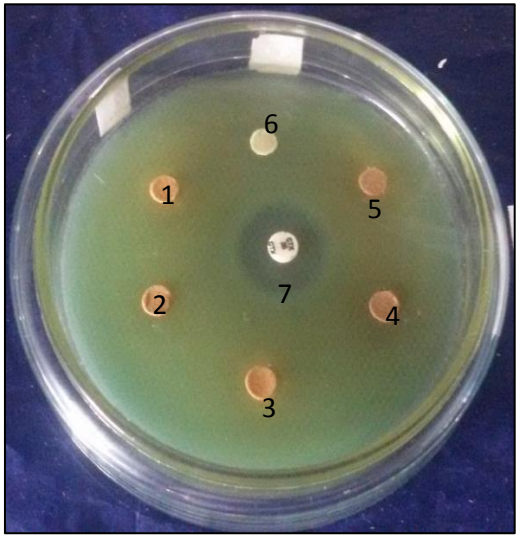
0 = No Inhibition

Antibacterial activity of ethanolic extract in disc diffusion method

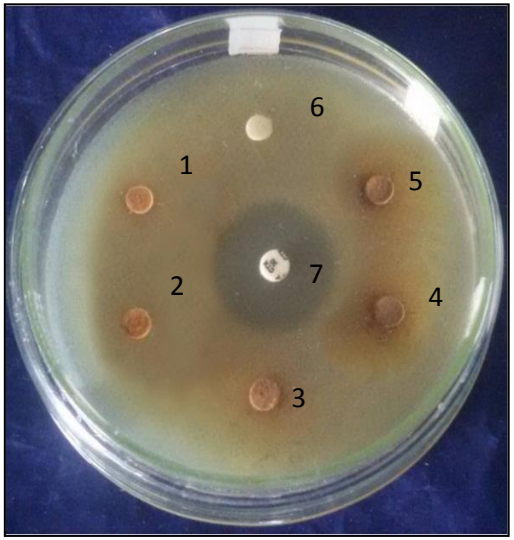
- The different concentrations of ethanolic extract, solvent control disc with ethanol and cefotaxime(30 µg/disc) were tested on three control strains of bacteria.



S aureus



P aeruginosa



E coli

1 = 1 mg/disc concentration
2 = 2 mg/disc concentration
3 = 3 mg/disc concentration
4 = 4 mg/disc concentration
5 = 5 mg/disc concentration
6 = Control disc, Distilled water
7 = Cefotaxime (30 µg/disc)

Figure 5. Antibacterial activity of ethanolic extract in disc diffusion method

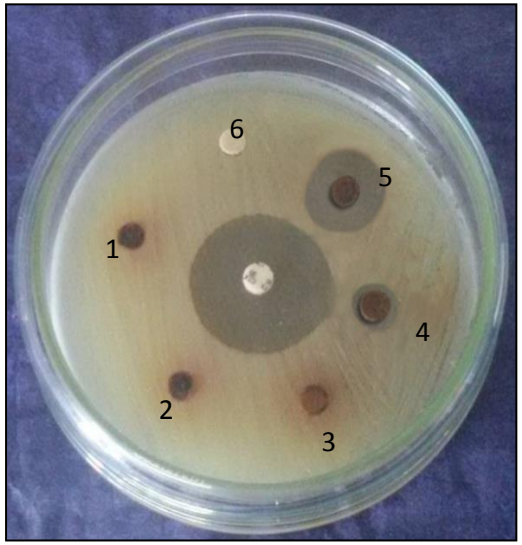
Table 2. Antibacterial Activity of Ethanolic Extract of roots of *S. cochinchinensis* Gagnep. for Disc Diffusion Method

Conc (mg/disc)	Zone of inhibition in mm		
	Staphylococcus aureus	*Pseudomonus aeruginosa*	*Escherichia coli*
1(mg/disc)	0 mm	0 mm	0 mm
2(mg/disc)	0 mm	0 mm	0 mm
3(mg/disc)	10 mm	0 mm	0 mm
4(mg/disc)	11 mm	0 mm	0 mm
5(mg/disc)	11 mm	0 mm	0 mm
Ethanol	0 mm	0 mm	0 mm
Cefotaxime (30 µg/disc)	27.5 mm	20 mm	26.5 mm

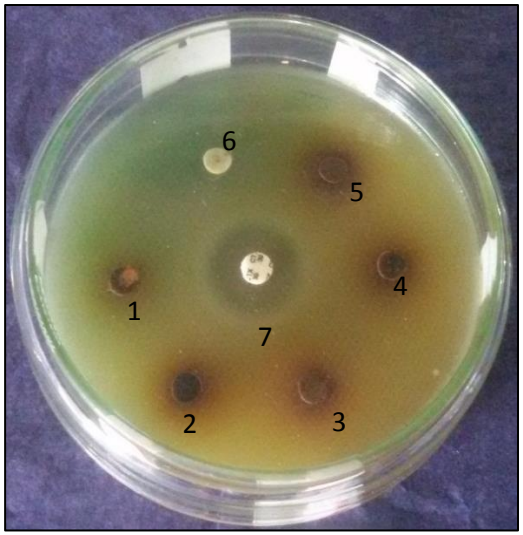
0 = No Inhibition

Antibacterial activity of methanolic extract in disc diffusion method

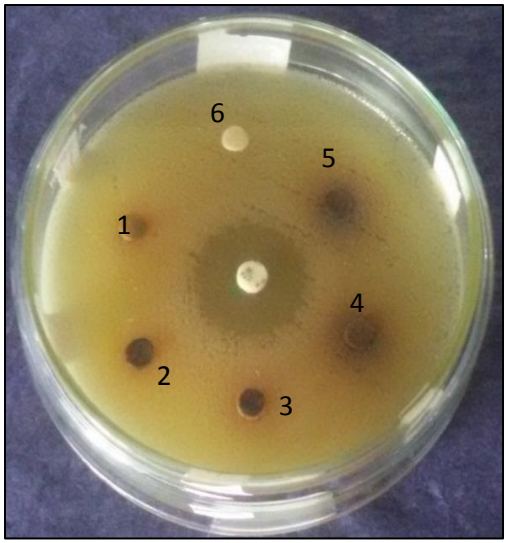
- The 4 mg/disc and 5 mg/disc of methanolic extract showed the diameters of zone of inhibition with 11 mm and 16 mm respectively.
- The average diameters of inhibition zone of standard drug, cefotaxime disc was 27.5 mm whereas no seen in solvent control disc.



S aureus



P aeruginosa



E coli

1 = 1 mg/disc concentration
2 = 2 mg/disc concentration
3 = 3 mg/disc concentration
4 = 4 mg/disc concentration
5 = 5 mg/disc concentration
6 = Control disc, methanol
7 = Cefotaxime (30 µg/disc)

Figure 6. Antibacterial activity of methanolic extract in disc diffusion method

Table 4. Antibacterial Activity of Methanolic Extract of roots of *S. cochinchinensis* Gagnep. for Disc Diffusion Method.

Conc (mg/disc)	Zone of inhibition in mm		
	Staphylococcus aureus	*Pseudomonus aeruginosa*	*Escherichia coli*
1(mg/disc)	0 mm	0 mm	0 mm
2(mg/disc)	0 mm	0 mm	0 mm
3(mg/disc)	0 mm	0 mm	0 mm
4(mg/disc)	10 mm	0 mm	0 mm
5(mg/disc)	16 mm	0 mm	0 mm
Methanol	0 mm	0 mm	0 mm
Cefotaxime (30 µg/disc)	33 mm	17 mm	24 mm

0 = No Inhibition

Comparison of zone of inhibition among three extracts in disc diffusion method

- The ethanolic extract showed antibacterial activity against *S. aureus* with the diameter of inhibition zone, 11 mm at the concentration of 5mg/disc (AI= 0.48).
- Similarly, the methanolic extract also produced 16 mm zone of inhibition against *S. aureus* at the concentration of 5mg/disc (AI = 0.40).
- Hence, ethanolic and methanolic extracts had antibacterial activity on *S. aureus*.

Discussion

- In concept of traditional medicine, Gone-Tha-Mya has the taste with hot, bitter and pungent.
- Nargathein (1971) described that the drugs which have the bitter taste can be used for the treatment of skin diseases, impetigo, wounds and burns.
- This plant was also used for various types of skin infections and wounds in the treatment guideline of the Khwe-Hsaung (Myat-Htun, 1971).
- According to the above facts, this plant can be assumed that it is likely to possess antimicrobial action which is used traditionally for treatment of wound infection.

- In disc diffusion method, the ethanolic extract inhibited the growth of *S. aureus* with the diameters of zones of inhibition 10mm at 3 mg/disc, 11 mm at 4 mg/disc and 5 mg/disc concentration respectively.
- Statistically, the increase in diameter of zone of inhibition was significant dose related antibacterial activity on *S. aureus* ($r = 0.892$, $p = 0.042$).
- In screening of methanolic extract, it can be observed that (4 mg/disc and 5 mg/disc) concentrations exhibited 10 mm and 16 mm zones of inhibition against the growth of *S. aureus*.
- Statistically, the significant dose related to antibacterial activity of methanolic extract on *S. aureus* was $r = 0.894$, $p = 0.041$.

- Therefore it is indicated that the increasing dose enhanced more significant antibacterial activity.
- Therefore, the ethanolic and methanolic extracts are potential antibacterial agent against gram positive bacteria and they can be used to treat wound infections caused by *S. aureus*.
- This findings of the present investigation will be helpful for traditional practitioners and this plant could be a source of new antibiotic compounds.

Conclusion

- The research deals with the phytochemical constituents and the antibacterial activity on *S. aureus*, *P. aeruginosa* and *E. coli* by using agar dilution and disc diffusion method.
- In the preliminary phytochemical analysis, the three extracts showed the combination of alkaloids, phenolic compound, polyphenols, flavonoid and tannins which contribute to antimicrobial action but flavonoids and phenolic compounds are absent in aqueous extract.
- In agar dilution method, the three extracts could not have been determined against all tested organisms up to concentration of 500 µg/ml.

- Thus, the minimum inhibitory concentration should be determined by using the purified active constiutents of various extracts of *S. cochinchinensis* Gagnep.
- In disc diffusion method, it was found that the ethanolic and methanolic extracts exhibited antibacterial activity against *S. aureus* with significant dose effect relationship ($r = 0.892$ and $r = 0.894$, $p < 0.05$).
- The methanolic extract can be assumed to be more active against *S. aureus* than ethanolic extract.
- In comparison between cefotaxime(30g) and extracts of *S. cochinchinensis*, the concentration of 5mg/disc of the ethanolic and methanolic extracts showed antimicrobial potential against *S. aureus* with AI = 0.40 and AI = 0.48 respectively.

- However, all three extracts did not show antibacterial activity on *P. aeruginosa* and *E. coli* in all tested concentrations.
- Thus, the findings revealed that roots of *S. cochinchinensis* Gagnep. was more active against gram-positive bacteria than gram-negative bacteria.
- It was concluded that the methanolic extract of the tuberous roots of *S. cochinchinensis* exhibited intermediate antibacterial activity as well as resistant antibacterial activity in the ethanolic extract on *S. aureus* at 5mg/disc concentration.

- Therefore, these studies could be scientifically proved for the antibacterial agent against gram positive bacteria and also confirmed the potential of this species for the treatment of bacterial diseases in addition to develop a new antimicrobial agent.

Suggestion

- The antibacterial activity of ethanolic and methanolic extracts of tuberous roots of *S. cochinchinensis* should be investigated with the higher dose.
- The antibacterial activity of the other various parts such as leaves, fruits of *S. cochinchinensis* Gagnep. and other various solvent extracts on different microorganisms should be investigated.
- The MIC of the pure active compound isolated from the ethanolic and methanolic extracts of *S. cochinchinensis* Gagnep. should be determined.

- Other pharmacological actions as anti-inflammatory and wound healing activities of *S. cochinchinensis* should be scientifically investigated.
- The acute dermal toxicity test of these extracts of tuberous roots of *S. cochinchinensis* Gagnep. should be investigated.
- The *in-vivo* antibacterial action of this plant parts should be scientifically proved on infected wounds of laboratory animals for the purpose of external applications such as ointments, creams.

ACKNOWLEDGEMENTS

- We would like to express our sincere gratitude to Director-General of Department of Traditional Medicine for allowing the paper presentation.
- We would like to thank to Dr. May Wint War, Assistant Director, Public Health Laboratory, Mandalay for supporting bacterial control strains.
- We would like to appreciate to Professor Dr. Khin Maung Lwin, Rector and Dr. Cho Cho Oo, Professor and Head, Department of Microbiology, University of Medicine, Mandalay for their kind permission in use of Departmental facilities for antimicrobial activity.
- We want to give special thanks to research scientist and staffs from Research Division, University of Traditional Medicine and staffs from Department of Microbiology, University of Medicine, Mandalay for their kind cooperation and skillful technical help.

References

နာဂသိန်(အရှင်)(၁၉၇၂)၊ ပုံပြဆေးအဘိဓါန်၊ ကျော်ဝင်းဆွေပုံနှိပ်တိုက်၊ တတိယတွဲ၊ ရန်ကုန်၊ ၂၂၁။

နာဂသိန်(အရှင်)(၁၉၇၁)၊ အခြေပြုဆေးအဘိဓါန်၊ မဂလာပုံနှိပ်တိုက်၊ တတိယတွဲ၊ ရန်ကုန်၊၁၇၆။

မြတ်ထွန်း(ဦး)(၁၉၇၁)၊ခွေးဆောင်ဆရာကြီး၏သမားစဉ်ကုထုံးကျမ်း၊မဂလာပုံနှိပ်တိုက်၊ရန်ကုန်၊ ၂၁၂။

Aye-Thida-Win(2014). Pharmacognostic study of *Stemona burkillii* Prain. PhD Dissertation.Department of Botany, Universityof Yangon, Myanmar.

Elkington, B. G., Southavong, B., Sydara, K., Souliya, O., Vanthanouvong, M.(2011). Biological evaluation of plants of Laos used in the treatment of tuberculosis in Lao traditional medicine. National Institute of Health. Pharm Biol, 2009 January: 47(1):26- 33.

Forbes, BA., Sahm, DF. and Weissfeld, AS. (2007). Beiley& Scott's Diagnostic Microbiology. 12th edition. St.Louis, Missouri.

Harborne, JB.(1998). Phytochemical methods. 3rded. Chapmen & Hall. London, UK.

Jaturong, L. (1985). Antimicrobial activity of *Stemona* compounds in root extraction. botany.sci.ku.ac.th /Bot Seminar/fullpaper/O-24.pdf.

Percival, SL. and Dowd, SE. (2010). *Pseudomonas aeruginosa*, Microbiology of Wounds. In: Percival, S and Cutting, K, editors. CRC Press. Taylor and Francis Group. London. New York. p.198.

Percival, SL. and Thomas, JG. and Williams, D. (2010). An Introduction to the World of Microbiology and Biofilmology. Microbiology of wounds. Percival, S. and Cutting, K., editors. CRC Press. Taylor & Francis Group, LLC. United States of America. p. 15-16.

Raaman, N. (2006). Phytochemical techniques. New India Publishing Agency. PitamPura. New Delhi.

Tiwari, P., Kumar, B., Kaur, MG. and Kaur, H. (2011). Phytochemical screening and Extraction: A Review, International Pharmaceutica Sciencia, vol 1(1):98-106.

Viji, M. and Murugesan, S. (2010). Phytochemical analysis and antibacterial activity of medicinal plant *Cardiospermum halicacabum* Linn. Journal of Phytology (2010), 2(1):68-77.

World Health Organization (WHO). (2007). WHO Country Cooperation Strategy 2006-2011, India. Supplement on Traditional Medicine. WHO Secretariat report on Traditional Medicine: Fifty-sixth World Health Assembly: Provisional agenda item 14.10.

THANK YOU